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Truths and Myths About the Determination of Molar Mass Distribution of Synthetic and Natural Polymers by Size Exclusion Chromatography

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ABSTRACT: This article discusses various aspects of the determination of molar mass distribution by means of size exclusion chromatography (SEC) in various application modes. The effects of erroneous specific refractive index increment (*dn*/*dc*), branching, column performance, and enthalpic interactions on the results obtained by different SEC techniques are discussed. Combination of SEC and a light scattering detector represents the most direct way to the molar mass distribution of all natural and synthetic polymers as it completely eliminates the need for column calibration and to a certain extent eliminates the dependence of the obtained results on some operational variables such as flow rate, temperature, or injected mass. A multiangle light scattering (MALS) photometer has become the most frequently used light scattering detector capable of determination of molecular size as another important polymer characteristic. This article contrasts SEC-MALS method with other application modes of SEC from the viewpoint of some frequent confusions and misunderstandings. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40111.

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INTRODUCTION

Size exclusion chromatography (SEC) is a special type of liquid chromatography that separates molecules according to their hydrodynamic volume (not according to molar mass).[1,2] A distinguished feature of SEC is the absence of interactions between the analyzed molecules and column packing. Although in real SEC analysis the interactions often occur, they are always undesirable and the goal is to find separation conditions (mainly solvent and column packing) where they are minimized. Since the introduction of SEC in the 60s of the last century, the method underwent a significant advancement of the hardware and methodology. The analysis time per one sample decreased from originally several hours to about 30–45 min with possibility of significantly faster measurements whenever requested.[3,4] Today's SEC represents the most frequently applied method of the determination of molar mass distribution of synthetic and natural polymers that almost completely replaced traditional methods of molar mass determination such as classical light scattering in batch mode, membrane and vapor phase osmometry, capillary viscometry, cryoscopy, and ebullioscopy. However, SEC has one serious limitation given by the fact that it is not an absolute method of molar mass determination, i.e., the molar mass is not related to any directly measurable physical quantity. Instead, the molar mass is measured indirectly from the relation between the logarithm of molar mass and elution volume usually called SEC calibration. For detailed description of solute retention in SEC, column calibration and processing the experimental chromatograms as well as of SEC columns and various detectors the readers can be referred to the second edition of classical SEC book.[5]

The molar mass distribution can be determined by means of SEC in three application modes: (i) conventional SEC with column calibration, (ii) SEC with universal calibration and an online viscometer, and (iii) SEC with a light scattering detector, nowadays mostly multiangle light scattering (MALS). The advantage of MALS over single angle light scattering detectors is given by the ability to yield molecular size simultaneously with molar mass and thus to allow the detection and characterization of polymer branching. The accuracy of the particular methods is sometimes clouded by false claims of some instrument manufacturers or by misunderstanding or ignorance of basic principles from the side of users. The purpose of this article is to discuss the pros and cons of the various application modes of SEC, point out possible sources of inaccuracies and clarify some frequent misunderstandings.

In SEC separation, the experimental chromatogram is broadened in the course of sample passage through the system of columns, interconnecting tubings, unions, and detector cells.[6]

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The broadening is caused by axial diffusion, lag time during the sample diffusion in and out of column packing, and whirling of the separated zones in the packed columns and other parts of chromatography set-up. Due to the peak broadening, a peak of an absolutely monodisperse sample is of different shape and broadness than a theoretical rectangular peak of the intensity proportional to the injected mass and the broadness equal to the injected volume. Despite peak broadening, for monodisperse samples, all molecules eluting across the peak are of identical molar mass. That is, the case of proteins and also of nearly monodisperse synthetic polymers prepared by sophisticated synthetic routes. For a polydisperse sample, the peak broadness is a superposition of the real broadness given by the molar mass distribution and the peak broadening. As a result of peak broadening, the molecules eluting within particular elution volume slices are polydisperse. Various methods of band broadening corrections have been published—the recent references may be useful to readers more interested in the band broadening issue.[7–11] However, using high performance SEC columns, the broadening effect can be neglected and the experimental data can be treated as if the particular elution volume slices were monodisperse. Neglecting the peak broadening brings significant simplification of processing the experimental data no matter if using calibration approach or light scattering detector. The comparison of molar mass averages determined by SEC or SEC-MALS with the values obtained by other methods of molar mass determination indicated that ignoring the band broadening really does not result in significant errors of molar mass averages.[12] As a matter of fact, SEC can potentially suffer from other more serious sources of inaccuracies that may result in several tens percent errors of molar mass or even bring completely confusing and misleading results.[13]

The most common application of SEC is the determination of molar mass distribution and molar mass averages. The molar mass is the most typical characteristic of polymers that not only differentiates them from other organic compounds, but which is responsible for many unique polymer properties. A typical feature of most of synthetic and natural polymers is polydispersity, i.e., the fact that a polymer sample consists of molecules of various molar mass usually spanning several orders of magnitude. In addition to polydispersity of molar mass, polymers can be polydisperse with respect to their chemical composition or architecture of polymer chain. The latter is mostly related to branching of polymer chains, which may occur purposefully in order to modify polymer properties or unintentionally due to various side reactions. The polydispersity of chemical composition concerns the analysis of copolymers, i.e., polymers synthesized from two or more monomers, when molecules of identical molar mass can consist of different fractions of monomer units. In many technically important polymers, the polydispersity of molar mass coexists with the polydispersity of chemical composition and/or branching.

THEORY AND BASIC PRINCIPLES

To understand the backgrounds behind various techniques and avoid false believes, we need to look at several basic relationships.

Characterization of Molar Mass Distribution by SEC

SEC data allow the calculation of molar mass averages commonly used in polymer science, in particular the number-average (M_n), the weight-average (M_w), and the z-average (M_z). They are determined using well-known equations:

$$M_n=\frac{\sum c_i}{\sum \frac{c_i}{M_i}},\quad M_w=\frac{\sum c_i M_i}{\sum c_i},\quad M_z=\frac{\sum c_i M_i^2}{\sum c_i M_i} \tag{1}$$

where c_i is the concentration of molecules (in g/mL) eluting within the ith elution volume slice and M_i is their molar mass. As the area of the ith elution slice of the chromatogram from a concentration sensitive detector, A_i is directly proportional to the concentration of eluting molecules ($c_i=\text{constant}\times A_i$), the concentration in absolute units is not necessary and the slice area A_i can be used in the above equations instead. However, the absolute concentration is necessary in the case of online viscosity and light scattering detectors because it is needed for the calculation of slice intrinsic viscosity and molar mass, respectively. The absolute concentration is mostly obtained from the signal of a refractive index (RI) detector using the following equation:

$$c_i=\frac{\alpha(S_i-S_{i,\text{baseline}})}{dn/dc} \tag{2}$$

where α is the RI detector calibration constant (in RI units per volt), S_i and $S_{i,\text{baseline}}$ are the RI detector sample and baseline signals expressed in volts, respectively; and the dn/dc is the specific RI increment. Alternatively, the concentration can be determined from the response of a UV detector assuming the extinction coefficient of polymer under investigation is known. An infrared detector, finding utilization mainly in the area of polyolefin characterization, can be mentioned as another type of concentration sensitive detector.

Note that in case of oligomers and copolymers, the detector response may vary across the chromatogram and the determination of correct concentration can only be achieved by using multiple detection.[14] The variation of dn/dc with chemical composition affects also the molar mass determined by a light scattering detector and true molar mass and structural information of heterogeneous copolymers can be obtained only after correcting for chemical heterogeneity.[15]

The molar masses M_i of molecules eluting at particular elution volume slices can be obtained from (i) calibration curve, (ii) universal calibration curve and intrinsic viscosity determined by a viscometer, and (iii) from the signal of a light scattering detector. The common assumption for all the methods is that the polymer fractions eluting from the set of SEC columns are monodisperse, i.e., the effect of peak broadening is negligible and the molar mass for each volume slice is just molar mass M_i and no average.

Note that the values of c_i (or A_i) and M_i allow not only the calculation of molar mass averages by means of eq. (1), but the determination of the differential and cumulative distribution curves as well.[5]

Column Calibration

Conventional Calibration. The relation between the molar mass M and elution volume V can be usually described by a third-order polynomial:

$$\log M = a + bV + cV^2 + dV^3 \quad (3)$$

The calibration curve can be easily established by the measurements of narrow standards, for which the elution volume of the peak can be related with the nominal molar mass. The most serious limitation of the calibration approach is that standards for few polymers are available. Polystyrene standards prepared by anionic polymerization are mostly used for the column calibration in the most frequently used organic solvent (tetrahydrofuran, THF), whereas dextran or pullulan standards are applied for aqueous solvents. Standard proteins are used to establish the calibration for the determination of molar mass of unknown proteins. However, for proteins, the calibration approach can be especially inaccurate due to significant configuration differences and high probability of enthalpic (e.g., ionic, hydrophobic) interactions with column packing.[16]

A real polymer sample requiring analysis is mostly of different chemical composition than the standards and the molar mass averages are solely apparent values, i.e., the values of a hypothetical polymer sample of the chemical composition identical with the calibration standards and the distribution of hydrodynamic volume equal to that of the analyzed polymer. It must be also emphasized that branching leads to strong errors in molar mass even in the case of calibration appropriate to given polymer kind; because branching reduces the molecular size[17] and thus the smaller branched molecules appear in conventional SEC as having lower molar mass.

Literature shows various other methods of the calibration of SEC columns by means of polydisperse standards or the recalculation of the calibration established with narrow standards to a calibration valid for a polymer of different chemical composition.[12,18–20] The common drawback of these methods is that they require something what is often unavailable or doubtful. Well characterized polydisperse standards are rare, and in addition, the calibration based on them may be less accurate than that based on narrow standards. The recalculation of calibration relation to a polymer of different chemical composition requires parameters of the Mark-Houwink equation. However, significantly different Mark-Houwink parameters can be found in the scientific literature for a given polymer and the reliability of the Mark-Houwink parameters may be often questionable due to the lack of experimental details on their determination. In addition, for many polymers, no Mark-Houwink parameters can be found in the literature. Moreover, the recalculation of calibration using Mark-Houwink parameters becomes inaccurate toward lower molar masses, because the parameters are constant only above a certain molar mass limit, which is somewhere between 10,000 and 20,000 g/mol.[12] The universal calibration may fail in case of oligomers as shown in Ref. 21. It must be emphasized that none of the calibration procedures can account for possible enthalpic interactions[13] of polymer molecules with column packing.

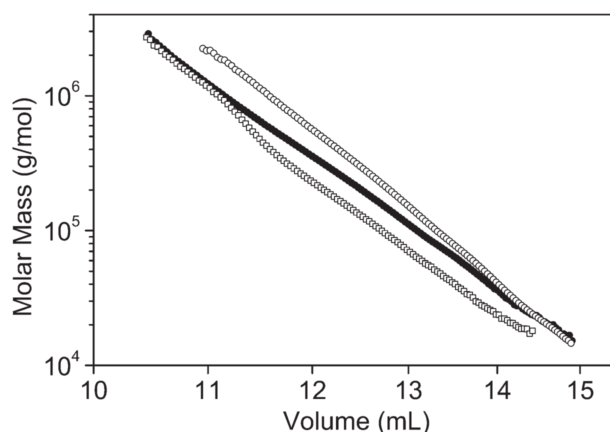

Figure 1. Molar mass–versus–elution volume plots of polystyrene (•), poly(vinyl chloride) (□), and branched polystyrene (○).

Unfortunately, the previous limitations are unknown or ignored by many SEC users and strongly inaccurate results are often generated and used for polymer characterization. Example of incorrect characterization by conventional calibration is shown in Figure 1, which compares molar mass versus elution volume plots for linear polystyrene, branched polystyrene, and poly (vinyl chloride). The plots of molar mass against elution volume obtained by means of the MALS detector represent conventional calibration curves. Glancing at Figure 1, one sees that the calibration based on polystyrene would significantly overestimate the molar mass of poly(vinyl chloride) in the region of lower molar masses, yet toward higher molar masses the calibration curve of poly(vinyl chloride) approaches that of polystyrene most likely due to presence of branched molecules. On the other hand, due to compact structure of branched macromolecules, the conventional calibration strongly underestimates the molar mass of branched polystyrene regardless of identical chemical composition. It is worth noting that Figure 1 gives only a slight touch of possible errors that can be generated by using incorrect calibration.

Another weak point of SEC with conventional calibration is a strong dependence of the obtained results on many experimental parameters, such as flow rate accuracy, injected mass, temperature, column type and performance, and quality and number of the calibration standards.[12] It is worth noting that solely one percent flow rate deviation from the flow rate used for column calibration results in noticeable errors of molar mass. Although the effect of flow rate fluctuations can be addressed by a flow marker, the flow rate remains absolutely key parameter for conventional SEC with column calibration.

Universal Calibration with Online Viscometer. The idea of universal calibration was introduced by Benoit and coworkers[22] who proved the product of intrinsic viscosity and molar mass ($[\eta]M$) to be a universal calibration parameter in SEC, i.e., the molecules eluting in a given elution volume have the same product $[\eta]_i M_i$ and the function:

$$\log([\eta]M) = a + bV + cV^2 + dV^3 \quad (4)$$

is identical for polymers of different chemical composition and/or branching.

The concept of universal calibration parameter is sound, because the product $[\eta]M$ is proportional to the hydrodynamic volume[23]:

$$V_h = \frac{[\eta]M}{2.5N_A} \tag{5}$$

and thus consistent with the major separation mechanism of SEC. In eq. (5), N_A is Avogadro's number and V_h is the volume of sphere that would have the same hydrodynamic behavior as a polymer molecule of given molar mass and intrinsic viscosity.

Equation (4) can be established by a series of narrow standards similarly as in the case of conventional calibration. The values of nominal molar mass and intrinsic viscosity determined by the online viscometer are used to establish the universal calibration. To get the molar mass distribution of unknown sample, the molar masses M_i are calculated from the values of $[\eta]_i M_i$ obtained from the universal calibration at particular elution volumes V_i and corresponding intrinsic viscosities $[\eta]_i$. The intrinsic viscosities $[\eta]_i$ are determined from the specific viscosities $\eta_{sp,i}$ measured by the online viscometer and concentrations c_i measured by the concentration sensitive detector:

$$[\eta]_i = \lim_{c \to 0} \frac{\eta_{sp,i}}{c_i} \tag{6}$$

At low concentrations, typical of SEC the concentration dependence of specific viscosity can be neglected and the ratio $\eta_{sp,i}/c_i$ is equaled to be the intrinsic viscosity at a given elution volume.

As evident from the above equations, the directly measured quantity is not the molar mass, but the intrinsic viscosity. The molar mass is determined from the universal calibration, which means the universal calibration procedure is of similar sensitivity to various experimental variables as the conventional calibration. Nevertheless, the universal calibration approach is based on sound physical principles and can provide correct molar masses assuming all operational variables are under control and there are no secondary non-SEC separation mechanisms. A certain disadvantage of the universal calibration is larger volume between the viscometer and the RI detector compared with the combination of MALS-RI, which significantly contributes to the interdetector peak broadening. The interdetector peak broadening causes that a given elution volume slice measured by a given detector is not exactly identical with the same slice when passing other detectors connected in the series and consequently affects the accuracy of molar mass and intrinsic viscosity. The effect of interdetector peak broadening can be compensated for by a so called band broadening correction that is embedded in ASTRA® software (Wyatt Technology Corporation).[24]

Light Scattering Detection

In contrast to the calibration methods described in the previous chapter, the combination of SEC with a light scattering detector completely eliminates the calibration step. The molar mass is determined directly from the intensity of scattered light at zero scattering angle. The basic light scattering equation describing the angular and concentration dependence of the intensity of scattered light is[25]:

$$\frac{R_\theta}{K^* c} = MP(\theta) - 2A_2 cM^2 P^2(\theta) + \ldots \tag{7}$$

where R_θ is the excess Rayleigh ratio, c is the concentration of polymer in solution (g/mL), M is the molar mass (weight-average M_w in case of polydisperse polymer), A_2 is the second virial coefficient, K^* is the optical constant, and $P(\theta)$ is the particle scattering function.

The Rayleigh ratio R_θ is the intensity of light scattered at an angle of observation θ related to the intensity of incident radiation and geometry of the light scattering instrument. The quantities primarily measured by the diodes of MALS detector are voltages, which are transferred to the Rayleigh ratios by means of instrumental constant determined by a standard liquid of well-known Rayleigh ratio (mostly toluene).

The optical constant K^* is defined for the vertically polarized incident light as:

$$K^* = \frac{4\pi^2 n_0^2}{\lambda_0^4 N_A} (dn/dc)^2 \tag{8}$$

where n_0 is the RI of the solvent at the incident wavelength, λ_0 is the incident radiation wavelength at vacuum, N_A is Avogadro's number, and dn/dc is the specific RI increment.

The particle scattering function $P(\theta)$ describes the decrease of the scattered light intensity with increasing angle of observation caused by the destructive interference of light beams scattered by different parts of the same macromolecule. It is defined as the ratio of the intensity of radiation scattered at an angle θ to the intensity of radiation scattered at zero angle:

$$P(\theta) = \frac{R_\theta}{R_0} \tag{9}$$

In the region of small angles, the angular dependence of the intensity of scattered light can be expressed as[26]:

$$\lim_{\theta \to 0} P(\theta) = 1 - \frac{16\pi^2}{3\lambda^2} R^2 \sin^2(\theta/2) \tag{10}$$

where λ is the wavelength at a given solvent and R is the root mean square (RMS) radius (R; z-average in case of polydisperse polymers, R_z). That means, the slope of the angular dependency at zero angle enables the RMS radius of macromolecules and particles to be determined. Note that for small polymer molecules with RMS radii below roughly 10 nm, which scatter light equally in all scattering angles, the RMS radius cannot be determined.

Originally, the light scattering experiments were performed in batch mode, when a polymer sample was prepared at several concentrations covering about an order of magnitude and the angular variation of the intensity of scattered light was measured for each solution. The batch experiments can be still useful for the characterization of samples for which the SEC separation is problematic (mostly shearing degradation or strong enthalpic interactions). However, more information can be obtained when a light scattering instrument becomes a part of SEC set-up. In SEC-MALS, the intensity of scattered light is measured during the entire sample run at multiple angles simultaneously. Then, the quantities R_θ, c, and M in eq. (7) becomes $R_{\theta,i}$, c_i, and M_i. The angular variations of the scattered

light intensity $R_{\theta,i}$ measured at regular time intervals are extrapolated to zero angle to obtain the values of $R_{0,i}$ that are used for the calculation of molar mass according to the following equation:

$$\frac{R_{0,i}}{K^* c_i}{}_i = M_i \quad (11)$$

Note that the second term of eq. (7) can be typically neglected because $2A_2cM << 1$, because typical values of A_2 and c are of the order of magnitude 10^{-4} mol mL g^{-2} and less than 10^{-4} g/mL. Because the second virial coefficient decreases with increasing molar mass and the high molar mass fractions elute at lower concentrations at the beginning of chromatogram, neglecting the second term does not result in significant errors of molar mass even in the region of very high molar masses. On the other hand, although the analysis of oligomers requires about 10–20 times higher injected mass and the A_2 of oligomers is mostly of the order of magnitude 10^{-3} mol mL g^{-2}, the low molar mass makes the second term even less serious for the accurate molar mass determination of various oligomeric compounds.

The light scattering detectors differ in the number of angles at which the intensity of scattered light is measured. Two-angle instruments represent an ultimate case, which, however, does not allow evaluating the goodness of the fit and such three angles can be considered as minimum number for reliable extrapolation. Single angle photometers measure the intensity of scattered light either at 90° (right-angle light scattering, RALS) or at very low angle (mostly 7°) at which the Rayleigh ratio is assumed to be $R_{0,i}$. The latter approach is called low-angle light scattering (LALS). The former one can be used for small polymers for which the angular dependence of scattered light intensity can be neglected, or combined with online viscometer and utilization of the Flory–Fox[27] and Pticzyn–Eizner[28] equations. The RALS-viscometry method estimates molar mass from $R_{90,i}$ and RMS radius from that molar mass and intrinsic viscosity. The estimated RMS radius is entered into the theoretical particle scattering function for linear random coils to get first estimation of $R_{0,i}$ and so on until the difference in the results obtained by two consecutive steps becomes insignificant. It is evident that the accuracy of the RALS-viscometry method strongly depends on the validity of the Flory–Fox and Pticzyn–Eizner equations for a polymer under analysis.

EXPERIMENTAL

Chromatography system consisted of a Waters Alliance 2695 Separations Module coupled with a MALS photometer DAWN® HELEOS™, a RI detector Optilab® T-rEX, and an online differential viscometer ViscoStar™ (all detectors from Wyatt Technology Corporation, Santa Barbara, CA). The data were acquired and processed using the light scattering software ASTRA® 6 (Wyatt Technology Corporation). The SEC separation was achieved using two PLgel Mixed-E or PLgel Mixed-C 300 × 7.5 mm columns (Polymer Laboratories, part of Agilent). The solvent was THF at a flow rate of 1 mL/min. The samples were prepared as solutions in THF in the concentration appropriate to expected molar mass. The injected volume was 100 μL. NIST 1476 polyethylene standard was measured using a PolymerChar high temperature SEC system coupled to HELEOS™ and PolymerChar viscosity and infrared detectors, 3× PLgel Mixed-B 300 × 7.5 mm columns, trichlorobenzene at 160°C and flow rate of 1 mL/min, injection volume 200 μL.

MYTHS ABOUT THE DETERMINATION OF MOLAR MASS

Myth 1: MALS Cannot Be Used for Polymers with Low Molar Mass

The application of MALS for the analysis of oligomers is important because of their numerous technical applications and such need for thorough characterization and quality control. In some older books of macromolecular chemistry, the reader can read that light scattering can be applied solely for polymers with sufficiently high molar mass of $M_w \approx$ 10,000 g/mol and more. This statement was certainly correct several decades ago before the advancements in electronics, optics, laser technology, membrane filters, SEC column technology, and data collection and processing. Today's light scattering detectors can detect oligomeric molecules down to just several hundreds g/mol and the light scattering is not limited to the high molar mass polymers any more.

The signal-to-noise ratio of the MALS detector is of primary importance when the MALS is applied to the oligomers. It must be emphasized that the electronic noise of modern MALS detectors is significantly below the noise that arises from chromatography columns. The noise originating from the eluent is caused by submicrometer dust particles from the mobile phase and/or shedding from the column packing. This source of noise can be substantially reduced by using online filters between the pump and injector, solvent prefiltration (needed mainly for aqueous solvents), and sufficiently long continuous flushing of SEC columns at a constant flow rate used for real measurements (several hours to several days). In addition, special light scattering columns specifically manufactured for the application with light scattering are available for example from Wyatt Technology Corporation or Polymer Laboratories (part of Agilent). Besides minimizing the effect of particles in the eluent, increasing the sample injected mass is another way of enhancing the signal-to-noise ratio. In contrast to high molar mass polymers, where injecting solutions with concentrations over ≈ 0.2–0.5% w/v may result in undesirable viscous fingering effects and/or promoting the shearing degradation, significantly higher concentrations can be injected in case of oligomers without negative impact on separation efficiency.

Myth 2: Viscometer Provides Better Signal for Oligomers than MALS

This myth is related to the Myth 1 concerning the signal intensity of light scattering for oligomers. According to eq. (7), the intensity of scattered light is directly proportional to the product of concentration and molar mass. The response of viscosity detector is proportional to the product of concentration and molar mass to the power of Mark-Houwink exponent. For short oligomeric chains that do not show Flory's self-avoiding behavior, one can expect the Mark-Houwink exponent a to be close to 0.5 and such the decay of the viscometric signal with decreasing molar mass is certainly less pronounced than in the case of

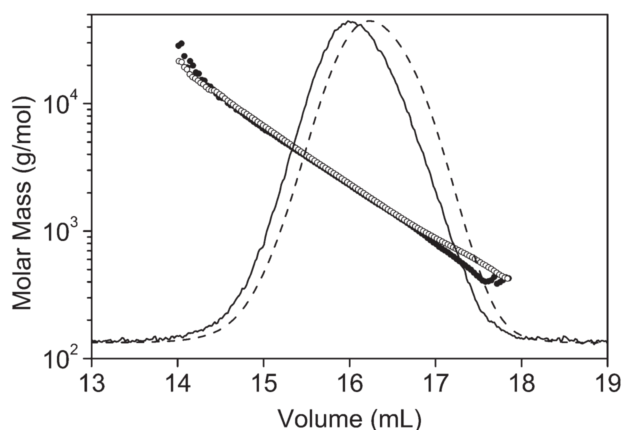

Figure 2. Molar mass–versus–elution volume plots for polybutylene glycol determined by MALS (•) and universal calibration (UC) with viscometer (○) overlaid with signals from MALS detector at 90° (solid line) and viscometer (dashed line). Injected amount 100 μL of 4% w/v solution in THF, $dn/dc = 0.068$ mL/g. M_n (MALS) = 1040 g/mol; M_n (UC) = 1170 g/mol. M_w (MALS) = 1650 g/mol; M_w (UC) = 1720 g/mol

MALS. However, this fact does not prove unsuitability of MALS for oligomers as demonstrated in Figure 2 where the molar masses and chromatograms obtained by the two techniques for a typical oligomeric sample are contrasted. The comparison shows surprisingly good agreement of molar mass plots and molar mass averages. Although the response of viscometer is slightly more intensive in the region of very low molar masses compared with the MALS detector, it obviously does not provide more information or more trustable results. Figure 2 addresses both Myths 1 and 2 and shows that reliable molar mass data and sufficiently intensive signal can be obtained by MALS detection down to few hundred g/mol molar mass range.

It may be worth noting in this context that the viscosity detector has lower signal intensity for dense macromolecules such as proteins and highly branched and hyperbranched macromolecules. In contrast, the light scattering detector has unmatched sensitivity to even trace amounts of compact species (highly branched macromolecules or aggregates) far below the detection limit of concentration detectors. The superior sensitivity of a light scattering detector to a trace level of ultra-high molar mass fractions is demonstrated in Figure 3 for a well-known branched polyethylene NIST SRM 1476. The chromatograms recorded by MALS, infrared and viscometric detectors show markedly different elution patterns at the very beginning of sample elution. The intensive MALS peak at the beginning indicates elution of a low concentration of species with very high molar mass. The high sensitivity of MALS to ultra-high molar mass species is given by the response being proportional to $M \times c$, whereas the concentration sensitive detector response is proportional solely to c. The low sensitivity of viscometric detector in this case is given by the response being proportional to $c \times M^a$ and the fact that the Mark-Houwink exponent a for highly compact branched species is close to zero (zero for solid spheres). Note that for highly compact sphere-like structures, for which a equals zero, the response of viscometer becomes independent of molar mass and is proportional solely to the concentration.

When comparing MALS and viscometric detectors, one should not forget that the light scattering signal is completely insensitive to flow rate and temperature fluctuations, whereas the viscometer signal is sensitive to flow rate irregularities and such perfectly working pulse free pump is needed for stable viscosity signal and good signal-to-noise ratio.

Myth 3: MALS Can Provide Only Weight-Average Molar Mass

This is absolutely true, but solely in case of MALS applied in the batch mode when the sample is measured without separation. In case of combination of MALS with a separation method, the experimental data allow easy determination of other molar mass averages as well as the molar mass distribution. The basic assumption is identical with that used in conventional SEC, i.e., the column band broadening is negligible and such the molecules eluting in a given time from the SEC columns are monodipserse, i.e., the light scattering detector measures just molar masses M_i and not the weight-average molar masses $M_{w,i}$. Although this assumption can never be entirely fulfilled, it does not seem to introduce significant errors in molar mass averages determined by SEC-MALS as can be seen from fairly good agreement of M_n values with the results from vapor phase osmometry[29,30] or membrane osmometry.[12] Nevertheless, due to the limited SEC resolution, the SEC with a MALS detector has tendency to overestimate the M_n and underestimate the M_z averages. However, it must be emphasized at this point that conventional SEC even with absolutely correct calibration has the opposite trend, i.e., underestimate the M_n and overestimate the M_z because of peak broadening. A significant advantage of SEC-MALS is that the weight-average is always correct even in case of excessive peak broadening or other effects affecting the SEC separation, because the weight-average M_w calculated from the weight-averages $M_{w,i}$ is always weight-average. The weight-average is determined based on the fundamental principle of light scattering and as a matter of fact it does not require any separation. In case of enthalpic interactions of the analyzed polymer with column packing conventional SEC or SEC with universal calibration and a viscometer provide completely incorrect information about the molar mass distribution including the value of M_w, whereas SEC with a

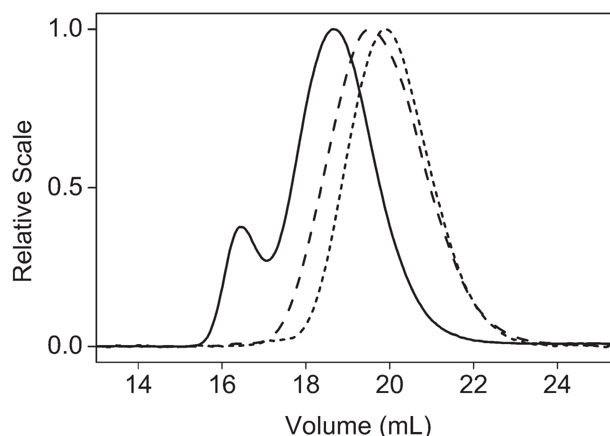

Figure 3. Chromatograms of NIST 1476 polyethylene standard recorded by MALS detector at 90° (solid line), viscometer (dashed line), and infrared detector (short dashed line).

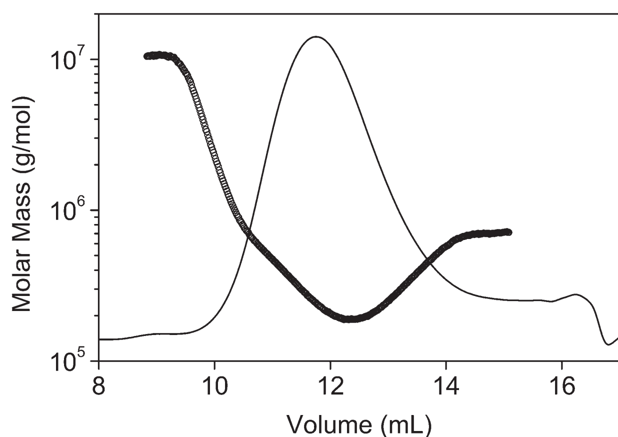

Figure 4. Molar mass–versus–elution volume plot typical for SEC affected by non-SEC separation mechanisms. RI chromatogram is overlaid here.

MALS detector yields at least correct M_w. In addition, the SEC-MALS data clearly reveal the non-SEC elution behavior from the course of molar mass–versus–elution volume plot. The plots showing even decrease of molar mass with elution volume indicate separation purely according to SEC separation mechanism, whereas molar mass plots parallel with the elution volume axis or the raise of molar mass with volume indicate non-SEC separation mechanisms. Example of elution behavior typical of non-SEC separation is depicted in Figure 4.

Myth 4: MALS Fails in Case of Heterogeneous Copolymers, Whereas Universal Calibration with Viscometer Yields Correct Results

Heterogeneous copolymers undoubtedly represent the most difficult and challenging area for the application of light scattering.[26] Average specific RI increment of a copolymer can be calculated according to simple relation:

$$dn/dc = w_A(dn/dc)_A + w_B(dn/dc)_B + \ldots \tag{12}$$

where w is the weight fraction of monomers in copolymer. However, the actual dn/dc values of the particular macromolecules differ according to their actual composition. In case of chemically heterogeneous copolymers, the elution volume slices may consist of molecules of identical hydrodynamic volume, but of different chemical composition and using the average dn/dc will result in the overestimation or underestimation of molar masses M_i. The chemical composition may vary along the elution volume axis and in ultimate case molecules consisting of only one monomer unit may elute in a given elution volume.

In contrast to the batch MALS experiment, where N % error in dn/dc results in 2 N % error in molar mass, the combination of eqs. (2) and (8) and (11) shows solely N % error in case of SEC-MALS. According to eqs. (2) and (6), the same error is obtained by universal calibration with viscometer when erroneous dn/dc is used. The exception would be less frequent cases when other concentration sensitive detector would be used instead of an RI detector. Then, the concentration would be obtained without need for dn/dc and in such particular case the dn/dc effect would be 2 N %.

The error of molar mass determined by a MALS detector or universal calibration with a viscometer depends on the polydispersity of chemical composition and on the difference of dn/dc values of parent homopolymers. If the dn/dc values of parent homopolymers are similar, such as in case of purely acrylic copolymers, the possible differences of dn/dc values and thus errors of molar mass are small, whereas for styrene–acrylic copolymers and other copolymers where parent homopolymers are of substantial different dn/dc the possible errors are more significant.

For the discussion of the effect of chemical heterogeneity on the molar mass, it may be worth noting that the polymerization process is often performed in the way to prepare copolymers chemically homogeneous, e.g., the monomer mixture is dosed continuously into the reactor over a certain period of time in order to minimize the effect of conversion heterogeneity, or copolymers are prepared by sophisticated polymerization techniques, such as for example anionic polymerization. That means for many technically important polymers, the heterogeneity problem may be sort of overrated.

Myth 5: MALS Is More Affected by Erroneous *dn/dc* than Universal Calibration with Viscometer

This myth has been already addressed in the previous paragraph. The above statement is correct, but solely for the batch MALS experiments. The dn/dc plays an important role because its square value contributes to the Rayleigh ratio in the light scattering, but in SEC-MALS dn/dc in the optical constant K^* is partly cancelled through the combination with the expression for the concentration and such the effect of dn/dc is exactly identical as in SEC with universal calibration, viscometer, and RI detector.

Myth 6: Low-Angle Light Scattering Is More Accurate than Multiangle Light Scattering

The fundamental difference of LALS from MALS approach is in the determination of $R_{0,i}$ for the calculation of molar mass using eq. (11). In LALS, the measurement is performed at low angle that is supposed to be zero, whereas in the MALS the intensities of light measured at multiple angles are extrapolated to zero angle. The former approach is sometimes claimed to be more accurate because it eliminates the extrapolation procedure, which is alleged as inaccurate. However, the data extrapolation is frequently used in various kinds of data processing in physics, chemistry, and other sciences, and there is no reason why this procedure should fail in the case of the determination of $R_{0,i}$. The ability of extrapolation to yield correct molar masses is demonstrated in Figure 5 that contrasts theoretical particle scattering function for linear random coils [eq. (13)][31] with the third-order polynomial fit through nine intensities corresponding to angles in the MALS photometer HELEOS^{TM} (Wyatt Technology Corporation).

$$P(\theta) = \frac{2}{x^2}(e^{-x} - 1 + x) \tag{13}$$

where

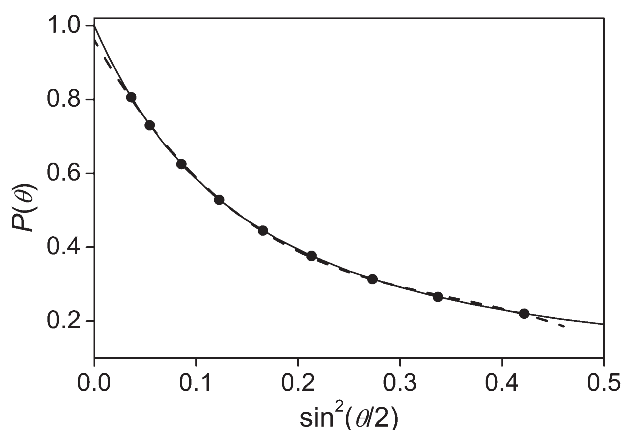

Figure 5. Comparison of theoretical particle scattering function (solid line) and extrapolation using the intensities at nine different angles in the range of 22°–81° (dashed line) for the random coil of RMS radius = 170 nm corresponding to the molar mass ≈ 10^7 g/mol. The intercept of 0.961 represents 3.9% error in the obtained molar mass. Third-order polynomial used for the extrapolation.

$$x=\frac{8\pi^2}{3\lambda^2}\langle r^2\rangle\sin^2(\theta/2) \quad (14)$$

where λ is the wavelength of the light in a given solvent and $\langle r^2\rangle$is the mean square end-to-end distance of the polymer chain.

It is obvious that the real and extrapolated lines coincide and the difference between the true R_0 and the value obtained by the extrapolation is negligible. It is worth noting that 170 nm random coils correspond typically to molar mass around 10^7 g/mol, i.e., far beyond the real molecules in most of synthetic and natural polymers. Note that the accuracy of the extrapolation increases with decreasing molar mass as the particle scattering function becomes less curved.

Another argument to support the LALS instruments is that the fit needed for the accurate extrapolation depends on molar mass. This is true as the plot of R_θ/K^*c against $\sin^2(\theta/2)$ becomes more curved with the increasing molar mass. This fact may complicate data processing of very broad polymer samples because of the need for different polynomial fit degrees at different parts of chromatogram. Nevertheless, alternative plots of K^*c/R_θ and $(K^*c/R_\theta)^{1/2}$ remain linear up to several millions g/mol. It has been shown[12] that even for very large molecules the molar masses are practically independent of the light scattering formalism and such plots of K^*c/R_θ or $(K^*c/R_\theta)^{1/2}$ can be recommended for processing the MALS data over very broad range of molar masses.

The LALS instruments *a priori* suffer from noise caused by submicrometer particles in the mobile phase that can never be completely eliminated. Because the intensity of scattered light decreases with increasing angle of measurement [see eq. (10)], these particles disturb the light scattering signal especially at very low angles, whereas the MALS approach completely eliminates the measurements at extremely low angles. The effect of submicrometer particles eluting from SEC columns is shown in Figure 6. Note that signal-to-noise ratio of chromatograms acquired at higher angles is well acceptable, whereas the polymer response at very low angle practically disappears in the signal noise. The noise becomes a serious issue when one needs to measure small polymers and oligomers and especially in the measurements in aqueous solvents where the noise level is always higher. In addition, the impossibility of acquiring the RMS radius data should be taken into account when considering the pros and cons of the MALS and LALS approach. The RMS radius becomes especially valuable when information about the molecular conformation and branching is requested. In addition, for large molecules, it is possible to learn about the molecular structure and conformation by studying the angular variation of scattered light intensity, and also the pattern of angular variation of the scattered light intensity can reveal coelution of small and very large molecules that may happen as a result of poor SEC resolution.

Myth 7: M_w Determined Using MALS Is Always Higher than M_w Determined with a Concentration Sensitive Detector

This myth can be understood in the following way: The sensitivity of light scattering detector increases with increasing molar mass, whereas the sensitivity of concentration sensitive detectors (e.g., RI) is molar mass independent and depends only on the concentration. For this reason, when broadly dispersed polymers (polydispersity >> 1) are analyzed, M_w determined using light

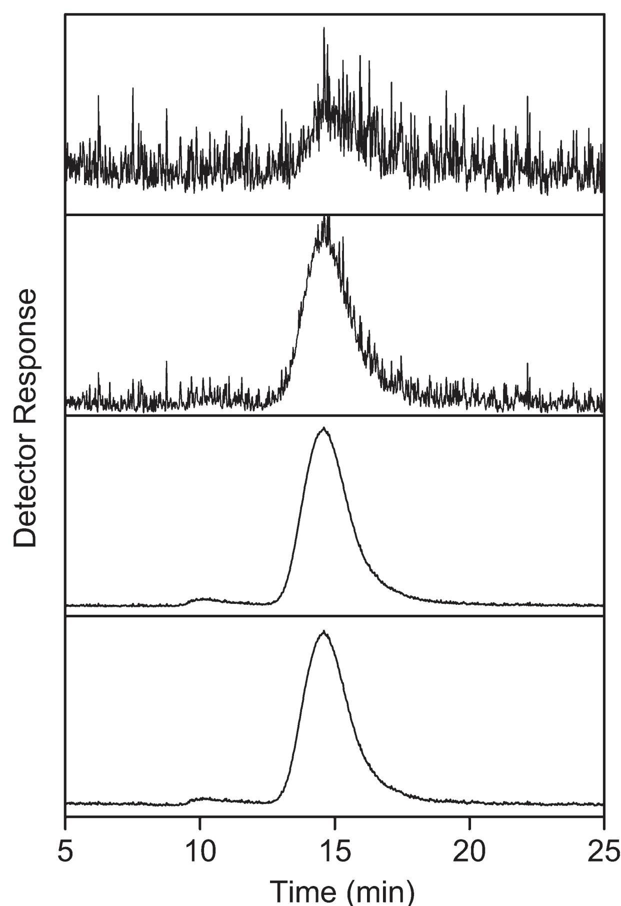

Figure 6. Chromatograms from SEC-MALS analysis of water soluble polymer of $M_w \approx$ 30,000 g/mol recorded at (from top to bottom) 15°, 45°, 90°, and 140°.

scattering results in higher values than M_w determined with a concentration sensitive detector.

The above is certainly not true and the myth arises from correct, yet misunderstood, difference of the response of the two types of detectors. As already explained, the MALS detector provides correct M_w even when SEC separation is affected by non-SEC separation mechanisms. The determination of correct M_w by SEC with calibration requires not only correct calibration for the polymer under analysis but also pure SEC separation unaffected by non-SEC separation mechanisms.

CONCLUSION

The most serious errors of molar mass obtained by MALS detector can be expected in case of heterogeneous copolymers when parent homopolymers are of significantly different *dn*/*dc*. However, the same errors are generated by universal calibration combined with online viscometer when RI detector is used as a concentration detector. Light scattering is no longer limited to high molar mass polymers, but with modern light scattering instrumentation reliable results can be obtained down to molar mass values of a few hundreds g/mol. In contrast to conventional SEC, the SEC-MALS results are not affected by long-chain branching of polymer chains. In combination with SEC, the MALS instrument does not yield only M_w, but other molar mass averages and the entire molar mass distribution as well. Due to the limited resolution of SEC separation, the SEC-MALS technique has a tendency to overestimate M_n and underestimate M_z, whereas SEC, no matter whether with conventional column calibration or universal calibration with viscometer, has the opposite trend as a result of band broadening in SEC columns. MALS provides unprecedentedly correct M_w even in case of SEC separation affected by non-SEC mechanisms. In addition, the non-SEC separation can be revealed from the plot of molar mass against elution volume. Significant advantage of MALS detection is given by low sensitivity to flow rate variations and other SEC operational parameters. The need to extrapolate the intensities recorded at multiple angles to zero angle does not represent a serious obstacle and the true advantage of the low-angle approach remains only hypothetical lying somewhere in the range of extremely large molecules. Not only that the multi-angle approach avoids measurements at extremely low angles that are most affected by the noise generated by particles in the mobile phase, but additional information can be obtained from the angular variation of the scattered light intensity, the RMS radius being the most important.

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