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***Abstract**:*

Size-exclusion chromatography (SEC) separates polymers by hydrodynamic volume (the universal calibration principle). Molecular weights can be determined using viscometry (relying on universal calibration) and light scattering (independent of universal calibration). In the case of complex branched polyacrylates with tetrahydrofuran as eluent, universal calibration is valid, although the separation in term of molecular weight is incomplete: a given elution slice contains a range of molecular weights, described in terms of a 'local polydispersity'. The local polydispersity index decreases when the number of branches per chain increases and complete separation is reached for highly branched chains.

Keywords: Multiple-detection Size-exclusion Chromatography (SEC), separation, polyacrylate, complex branched polymer, molecular weight, hydrodynamic volume

Introduction

Size-exclusion chromatography (SEC) is a technique widely used to analyze synthetic and natural polymers [1,2]. These polymers have a distribution of molecular weights (MW). Branched polymers constitute an important class of polymers, which can be divided in two main categories: (a) regularly-branched polymers (e.g. regular stars, combs, dendrimers, H, pom-pom polymers etc.[3]), in which all polymeric chains have the same number of branches and the branches have the same size for each chain; (b) complex branched polymers, in which there is a distribution of number of branches and/or size of the branches. Branching is present in several important families of polymer: (i) starch, the major component of human nutrition [4], also extensively used industrially [5]; (ii) glycogen, another energy storage polysaccharide [6]; (iii)

polyethylene, the most widely produced synthetic polymer, which is statistically branched when produced by free-radical polymerization [7]; (iv) poly(vinyl acetate), extensively used for paints and adhesives [8] and (v) polyacrylates, with numerous applications for coatings and pressure-sensitive adhesives [9]. Furthermore, all monomers polymerizable by chain polymerization can form complex branched polymers if copolymerized with a difunctional monomer (see e.g. [10,11]). Another family of interesting complex branched polymers comprises dendritic ones [12], but these will not be further considered in the present context. This work deals with statistically branched homopolymers: the case of copolymers, whose chemical heterogeneity can be another source of complexity, or the case of non-statistical branching are not treated here.

It is well known, but often unappreciated by practitioners, that even under ideal conditions, SEC separates polymer chains by their hydrodynamic volume V_h (universal calibration principle), but not by their MW. The validity of universal calibration for the separation of complex branched polymers has been the object of successive controversies in the literature. Wild and Guliana [13] studied fractionated polyethylene samples by SEC, viscometry, osmometry and light scattering. They observed that different calibration curves ($\log M = f(t_{el})$, where t_{el} is the elution time) were obtained for SEC of linear and long-chain branched polymers and concluded that long-chain branching plays a role in the SEC separation. Benoit *et al.* [14-16] demonstrated for a range of polymers that for each t_{el}, the product of intrinsic viscosity $[\eta]$ by molecular weight M is independent of the nature of the polymer, which constitutes the universal calibration in SEC. In practice, they measured weight-average intrinsic viscosity $[\eta]_w$ and weight-average molecular weight $\overline{M}_w$ of linear and different types of branched (comb, star) narrow standards, obtained by living anionic polymerization and/or fractionation. Universal calibration has been proved to be valid for many other polymers, e.g. polyethylene [13,17], poly(ethylene oxide) [18], polysaccharides [18,19], polypeptides [20], globular proteins [19], and hyperbranched polymers when the end-groups are have no significant interactions with the stationary phase [21,22]. The use of the product $[\eta]_w \cdot \overline{M}_w$ to apply universal calibration was disproved by Hamielec *et al.* [8,23-25] in the case of statistically branched poly(vinyl acetate). Their experimental results and theoretical treatment proved that universal calibration is actually valid for statistically branched polymers using the product $[\eta]_w \cdot \overline{M}_n$, where $\overline{M}_n$ is the number-average molecular weight. When the separation obeys a size-exclusion mechanism, a given t_{el} always corresponds to a single value of the product $[\eta]_w \cdot \overline{M}_n$, which has dimensions of molar volume and is the functional definition the hydrodynamic volume V_h.

As made clear by Hamielec and coworkers, complex branched polymers elute in SEC into fractions which are polydisperse in terms of MW even though they possess the same V_h [8,23-25]. The presence of more than one type of polymer molecule at a particular t_{el} has been termed "imperfect resolution" [24], "structural polydispersity" [11] or more generally "local polydispersity" [2,26]. Local polydispersity is generally assumed to be negligible [11,27]. Mueller *et al*. [21] proved the absence of local polydispersity in the SEC of hyperbranched poly(methyl methacrylate) using multiple detection. Balke *et al.* [28] characterized complex branched polymers by universal calibration together with low-angle laser light scattering (LALLS). They compared the three methods then available in the literature: chromatogram comparison, conventional calibration and universal calibration. This permitted the detection of the local polydispersity for some samples, but no quantification. All of these methods require the use of a linear equivalent of the branched sample. Synthesizing a linear equivalent over an appropriate range of MW may be expensive, time-consuming or even impossible, for example in the case of amylopectin. Furthermore, comparison methods are time-consuming. For a complex branched polymer, there can be a distribution of chains with different MWs but the same V_h. The separation will thus be incomplete even if

universal calibration is valid. Hence, SEC of a complex branched polymer cannot, even in principle, yield a molecular weight distribution (MWD), irrespective of whatever battery of online detectors may be employed.

SEC separation of some complex polymers has been claimed to be disturbed by anomalous elution behavior: the molecular weight determined by multi-angle light scattering (MALLS) at each t_{el} decreases with t_{el}, as expected, but then increases at higher t_{el} [29]. This was observed for star copolymers of styrene and butadiene [30], copolymers of styrene and divinyl benzene [30-32], heparin [33], dendritic polymers [34] and polymacromonomers [35]. It was also observed for linear chains, polystyrene (see figure 2 of [36]), poly(styrene sulfonate) (see figure 23 of [37]) and polyesters[38]. In the case of polyesters, the anomalous behavior is observed only when determining the molecular weight by MALLS but not with a determination by triple detection (combining right-angle laser light scattering and viscometry). Contradictory observations have been given for this effect such as the suppression of microgels because the presence of a precolumn may [30] or may not [32] mask anomalous elution behavior. Anomalous elution behavior is indeed not observed for hyperbranched poly(methyl methacrylate) analyzed by viscometry (relying on universal calibration) as well as light scattering [21,29]. Burchard *et al.*[6] also observed this anomalous behavior of the determined molecular weight against t_{el} with SEC MALLS of glycogen; however, they showed by successive degradation of glycogen that this effect is purely an artifact due to data treatment. Calibration curves log$M$ against t_{el} are meaningful only in the range where light scattering and refractometer signals are intense enough (in terms of signal-to-noise ratio). This has been proved by simulating SEC MALLS results including noise on the refractometer and MALLS detector signals [39]. At high t_{el}, at which the actual signal intensities are low, the noise induces the generation of successively negative and positive values of MW. However, these data are fitted as log$M$ by commonly used software and the logarithm of a negative number is not defined; such software thus replaces the negative values by zero before fitting. log$M$ values are then overestimated, even if a proper size-exclusion mechanism takes place. Note that the input chromatograms used to simulate “anomalous elution” (as shown by the refractometer and MALLS traces in figure 2 of [39]) seem to exhibit very little noise.

A summary of the literature on multiple-detection SEC of complex branched polymers follows. First, “anomalous elution” has been assigned to artifacts during data treatment and not due to branching. Second, conditions where universal calibration is valid for complex branched polymers have been found with a potential significant local polydispersity as demonstrated by the pioneering work from Hamielec. Local polydispersity indices significantly higher than unity have never been reported. Comparison of the molecular weight versus t_{el} determined both with viscometry (relying on universal calibration) and light scattering is particularly valuable to reveal the mechanism of separation of complex polymers [21,29]. Very few studies combine state-of-the art detection, viscometry and light scattering, together with appropriate data treatment to study the separation of complex branched polymers.

The mechanism of separation of complex branched polyacrylates by SEC is studied in this work using multiple-detection and a novel data treatment. Both viscometry and light scattering detectors are used and the molecular weights against t_{el} so determined are carefully compared. The aim of this article is first to investigate whether universal calibration is valid for the separation of complex branched polyacrylates. When universal calibration is valid, the mechanism of separation is then further examined, since complex branched polymers have not only a distribution of molecular weights (MW) but also distributions of long-chain branch lengths and topologies.

Experimental section

Polymerizations

Materials for polymerization and polymerization conditions are fully described in the supporting information. Poly(2-ethylhexyl acrylate) was synthesized by photopolymerization using a pulsed laser as described previously [40]. A 1-mL solution of 2-ethylhexyl acrylates/toluene 50/50 wt% was polymerized to a monomer conversion of 2.7 % (gravimetry). The amount of polymer was too small to allow determination of branching level by NMR, but poly(n-butyl acrylate) produced under similar conditions (pulsed UV lamp) were measured by ^{13}C solid-state NMR to contain 0.3 % of branches per monomer unit [41]. Poly(ethyl acrylate) and poly(methyl acrylate) were produced by conventional free-radical polymerization as a 4.7 mol·L^{-1} monomer solution in toluene to 99 % monomer conversion (gravimetry) [42]. ^{13}C NMR measurements were done in $CDCl_3$ at 125 MHz, with a 10 s relaxation delay and 20 000 transients, at 33 °C for PMA and 25 °C for PEA. They showed that respectively 2.1 ± 0.5 and 1.6 ± 0.5 % of the monomeric units are branched, not distinguishing between long- and short-chain branching [42]. The relative insensitivity of the solution-state NMR was overcome by the use of solid-state NMR,[42] inspired by a work by Klimke et al. [43]. Two poly(*n*-butyl acrylate)s were obtained by nitroxide-mediated radical polymerization. High conversion poly(*n*-butyl acrylate) was obtained by miniemulsion polymerization targeting high MW (theoretical $\overline{M}_n$ of 1.07×10^5 g·mol^{-1}, 88 % conversion). Another poly(*n*-butyl acrylate) sample was obtained by bulk polymerization targeting low MW (2.52×10^4 g·mol^{-1}, 80 % conversion). Two poly(*n*-butyl acrylate)s were a generous gift from Prof. Axel H.E. Mueller (University of Bayreuth) and were synthesized by anionic polymerization in THF in presence of lithium 2-methoxyethoxide as a ligand [44].

SEC set-up

Molecular weights for polyacrylates were determined with a Viscotek triple-detector SEC set-up composed of an online degasser, pump, manual injector, one precolumn and three columns (two mixed-C and one 10^2 Å, particle size of 5 μm) from Polymer Lab., the TDA (triple detector array, including in series RALLS (right-angle laser light scattering), LALLS at 7° (laser wavelength 670 nm), refractometer and finally viscometer), and Trisec® 2000 software. The software corrects for inter-detector delay and makes partial correction for band broadening; the related constants are given in the supporting information (Table 3) but the theory used by the software is not given by the supplier. The eluent was THF at 40 °C and 1 mL·min^{-1}. Toluene was used as a flow rate marker. As recommended by Coote and Davis [45], the injection loop was calibrated by weighing with toluene: its volume was 53.6 μL (arithmetic mean from 3 values: 53.1, 55.5 and 52.3 μL). The different detectors were calibrated injecting different polystyrene standards (PSS and Viscotek, Supporting Information Table 1 and 2, molecular weight at the peak and weight-average molecular weight were provided by the suppliers) of known concentration to calculate the mass constant of the refractometer, the constant of the viscometer and constants for each of the two light scattering detectors. On each day of use, the SEC set-up was checked using polystyrene, poly(methyl methacrylate) or poly(*n*-butyl acrylate) standards (Supporting Information). Eluent alone was regularly injected to check that the baseline of all the detectors, including light scattering, remained flat and that thus no bleeding of the columns could change the signal of the online detectors.

The d*n*/d*c* values used in this work were either determined using the refractometer or extrapolated from the literature in the case of poly(n-butyl acrylate) (see Supporting Information).

All averaging of curves, fits, etc. were carried out using Origin® software. In this work an online viscometer was used and thus the Mark-Houwink-Sakurada relation was not needed.

Optimization of multiple-detector SEC to reduce noise (case of polyacrylates)

It is important to optimize the concentration of the injected polymer to obtain low-noise chromatograms when using multiple-detector SEC. The uncertainty arising from the determination of the concentration by the refractometer will affect the determination of the intrinsic viscosity by the viscometer and/or the determination of the molecular weight by light scattering. This problem is enhanced by the difference in sensitivity between the refractometer, a mass-sensitive detector, and the molecular-weight sensitive detectors (viscometer or light scattering). The refractometer has a poorer sensitivity for high MWs and a higher sensitivity for low MWs than the molecular-weight sensitive ones. This explains for example some so-called "anomalous elution" behavior [6]. Optimizing the injection concentration in the case of polyacrylates is even more important because of their low refractive index increment d*n*/d*c* (around 0.06 $dL \cdot g^{-1}$ in THF, Supporting Information). Using the following empirical relation, the concentration C_{max} to be injected was calculated as [46]:

$$C_{max} = \frac{0.2}{[\eta] \cdot V_{inj}} \quad (1)$$

where C_{max} is in $g \cdot L^{-1}$, the intrinsic viscosity $[\eta]$ is in $dL \cdot g^{-1}$ and the injected volume V_{inj} is in mL. Note the unusual units of this empirical relation implying that the constant 0.2 includes a correction factor (10^4) for converting volumes into L and has dimension of volume (L). To prevent any overloading of the columns, we chose to work at concentrations of ca $C_{max}/2$ (typically 1 to 10 $g \cdot L^{-1}$). The resulting raw chromatograms had low noise (Fig. 1). They also give a qualitative indication of the presence of long-chain branching: for the very high MWs (several million $g \cdot mol^{-1}$, corresponding to the exclusion limit of the columns) at very low t_{el}, a very intense peak appears on the light scattering trace while nearly no signal can be detected by the refractometer. The same types of chromatograms have been observed for long-chain branched polyethylene [47].

Note that the determination of the concentration by the refractometer is still valid with statistically branched systems, since d*n*/d*c* has been shown not to vary with branching level [48]. The analysis of complex branched polymers by multiple-detection SEC is easier than that of copolymers with composition distributions; in the latter case, d*n*/d*c* indeed usually varies with composition.

Even working at the optimal injection concentration, the MWDs determined from these chromatograms are still very noisy (Fig. 2, gray line). Note that the highest level of noise lies on the y-axis, w(log*M*), since this is calculated using the refractometer trace divided by the slope of the calibration curve, $\log M = f(t_{el})$ [49]. The Trisec® software available with the device used here only allows the determination of MWDs via a regression fitting of the log*M* against t_{el} for one sample at a time. With our samples, this leads to very poor fits (Fig. 3 top). An improved method of data treatment is as follows. Instead of smoothing the data, Bruessau [50] recommends repeating the analysis several times and co-adding the chromatograms. Since we use a triple-detector SEC, we decided not to co-add the chromatograms but instead to co-add the $\log M = f(t_{el})$ obtained from these chromatograms. First, Trisec® is used to obtain the $\log M = f(t_{el})$ from the chromatograms of single injections using either universal calibration, triple detection or LALLS. It is assumed here that samples of the same monomer polymerized over the same narrow ranges of temperature (± 5°C) and monomer concentration (± 0.5 $mol \cdot L^{-1}$) will have similar branching distributions. The log*M* =

$f(t_{el})$ curves for all these polymers are plotted together and the total data set fitted by a polynomial. The arithmetic mean is thus calculated before the fit (Fig. 3 bottom). Fitting the $\log M = f(t_{el})$ curves for several samples brings two benefits: it reduces the noise, especially at the limit volumes on both sides, and the $\log M = f(t_{el})$ plot can be obtained over a broad range of MW by putting together samples having different MW ranges. The fit yields the calibration curve subsequently used to treat the refractometer chromatograms via a classical conventional calibration with Trisec®. The distribution so obtained is much less noisy (Fig. 2).

This two-step data treatment appears important for the determination of average MWs even in the case of linear polymers. Indeed, to obtain meaningful MWs in multiple-detector SEC and avoid so-called "anomalous elution" behavior, the integration limits have to be restricted to that part of the chromatogram where all the detectors yield a significant signal to prevent random number generation (i.e. number whose uncertainty becomes larger than the number itself) at low and high t_{el} (corresponding to very weak signals with the refractometer and with the MW sensitive detectors respectively) [51]. These integration limits would thus yield an overestimate of $\overline{M}_n$ (the integration limit at high t_{el} cuts out some oligomers) and an underestimate of $\overline{M}_w$ (the integration limit at low t_{el} cuts out the highest molecular weight) [50], and thus an underestimate of their ratio, the polydispersity $I_p = \overline{M}_w / \overline{M}_n$. Our two-step approach makes it possible to constrain the integration limits to a reasonable range to calibrate the columns using multiple-detection SEC. In the second step, conventional calibration is used with broad integration limits corresponding to the full refractometer signal for the whole polymer, including for example a low-MW tail where the light scattering signal is low.

Results and discussion

Comparison of the molecular weights determined by viscometry (relying on universal calibration) and light scattering using multiple-detection SEC

Multiple-detector SEC set-ups allow MWs to be determined by two independent techniques: viscometry (relying on universal calibration) and light scattering (LALLS or triple detection or MALLS). These techniques should yield the same MWs, and this is indeed seen within experimental error for all analyzed standards, which are linear chains: polystyrene, poly(methyl methacrylate) and poly(*n*-butyl acrylate) (Supporting Information and Fig. 4). The standards synthesized by anionic polymerization, polystyrene, poly(methyl methacrylate) and two poly(*n*-butyl acrylate) samples (Fig. 4 b), are likely to be linear chains, since no branching or transfer to polymer has been reported in the literature with this polymerization method [52]. Analogs of the poly(*n*-butyl acrylate) produced by controlled free-radical polymerization (Fig. 4a) were studied by MALDI TOF MS, which detected short-chain branching but no long-chain branching [53]. Short-chain branching has a very limited effect on the V_h as long as only a few percent of the monomer units are branched [54]. The results obtained with the standards prove that the SEC set-up is well calibrated, and also that universal calibration is valid with the columns, eluent and poly(alkyl acrylates) used here.

When statistically branched polyacrylates were analyzed, the MW obtained from universal calibration was significantly lower than that from the LS-based techniques. In the case of polyacrylates, three different free-radical polymerization processes were compared: PLP, conventional free-radical polymerization and nitroxide-mediated controlled free-radical polymerization. The same samples were injected several times and the raw $\log M$ against t_{el} are presented in Fig. 5. Differences in the $\log M$ against t_{el} determined by viscometry/universal

calibration and light scattering-based methods have so far been reported only twice in the literature by Hofe in the case of SEC of polyisocyanates in hexane [55] and by Mourey (nature of the polymer not specified) [29]. The difference between MWs determined by universal calibration and light scattering can be due to non-size-exclusion effects, i.e. adsorption effects. The recovery is thus an important parameter: for polyisocyanates in hexane, it is low, as only 30-50 % of the injected polymer is detected. The mechanism of separation is thus certainly not a pure size-exclusion one and universal calibration is not valid in these conditions in hexane. In the case of the polyacrylates analyzed in this work, THF is an appropriate solvent for SEC separation of flexible polyacrylates, since recovery was measured to be quantitative. The difference between MWs could also be due to the presence of microgels. However, the microgels would affect the MWs determined at the lowest elution volume and the difference is not significant at these elution volumes. Furthermore, microgels are obtained by intermolecular transfer to polymer combined with termination by combination. The amount and nature of microgels likely differs significantly among the three different polymerization process used in this work, but the difference in MWs is qualitatively the same for all the samples.

Why does universal calibration yield lower molecular weights than light-scattering based techniques?

The difference between MW determined by universal calibration and triple detection is absent for linear chains and systematic for branched acrylic polymers and thus very likely arises in this case from the presence of distributions of lengths of long-chain branches and of number of branches per chain in this case. Each elution slice then contains a range of molecular weights, as in the "incomplete separation" in terms of MW observed for statistically branched poly(vinyl acetate) by Hamielec *et al.* [8,23,25] At a given t_{el}, a mixture of chains of different MWs will be detected. In-line viscometry yields the local weight-average intrinsic viscosity and this value used together with the universal calibration curve yield the number-average MW of this iso-V_h distribution: $\overline{M}_n(V_h)$ [8,23,25,49]. On the other hand, every light-scattering-based method will yield a weight-average MW of this iso-V_h distribution: $\overline{M}_w(V_h)$ [8][56][49]. This is consistent with our experimental observation that universal calibration yields lower MWs than triple detection ($\overline{M}_n(V_h) < \overline{M}_w(V_h)$). Note that the average MWs for the iso-V_h distribution are completely different from the average MWs for the whole MWD: $\overline{M}_n(V_h) \neq \overline{M}_n$ and $\overline{M}_w(V_h) \neq \overline{M}_w$. The measurement of the dependence of both $\overline{M}_n(V_h)$ and $\overline{M}_w(V_h)$ on V_h makes it possible to measure the local polydispersity index $I_p(V_h) = \overline{M}_w(V_h) / \overline{M}_n(V_h)$ [24,25]. Experimental values of $I_p(V_h)$ are reported for the first time in this work. The values for the different polyacrylates studied are given in Fig. 6 and some are significantly higher than unity, as predicted by Hamielec *et al.* [8] Thus local polydispersity is not always negligible. The values, up to 2.5, are reasonable when one considers that at a given intrinsic viscosity, the molecular weight can change by several orders of magnitude depending on branching (up to 2 orders of magnitude for copolymer of methyl methacrylate and ethylene glycol dimethacrylate [10]).

Validity of the local polydispersity observed for slightly long-chain branched polyacrylates

The occurrence of a local polydispersity was predicted by Hamielec *et al.* [25] and proved by some of the present authors [49] through a rigorous derivation of the form of the response from different SEC detectors. The occurrence of an artifact due to unknown technical issues was ruled out by applying several consistency checks: (i) high polydispersity is observed for different types of samples produced by conventional free-radical polymerization (Fig. 6a), controlled radical

polymerization (Fig. 6b) and pulsed laser polymerization (Fig. 5c); (ii) linear polymers, even of the same chemical nature, do not exhibit such a polydispersity (Fig. 4); (iii) the same results were obtained with the same SEC set-up but over an extended period of time using different calibrations for the detectors and the columns (Supporting Information). The SEC columns used in this work yielded a good separation (an internal test was the regular analysis of multimodal samples produced by pulsed laser polymerization [57]).

MALDI ToF mass spectrometry used as an offline or online detector [58] might, in principle, provide another means to measure the local polydispersity index. However, MALDI ToF mass spectrometry suffers from bias in molecular weight and thus cannot be used to measure polydispersity [58,59] unless this were close to unity. MALDI ToF mass spectrometry would underestimate the local polydispersity index and is thus unlikely to confirm or disprove the occurrence of local polydispersity. Fractionation by SEC is generally used to overcome the bias in MALDI ToF MS, but in the case of our complex branched polymers, the fractionation gives insufficient separation in molecular weight to avoid this bias.

Under similar conditions to those used for polyacrylates (complex branched polymers, constant d*n*/d*c*, Fig. 5 d)), Balke *et al.* [26] predicted a different behavior: the difference between universal calibration and LALLS would decrease with t_{el}, if universal calibration is valid. The difference with our results (Fig. 5 a, b and c) can be explained by the branching distributions. Balke injected polyesters obtained by a different process, namely polycondensation, and the branching distribution is obviously different from that obtained by a free-radical polymerization process.

The observation that local polydispersity index $I_p(V_h)$ can be significantly greater than unity could also be due to band broadening, even in the case of linear chains [25]. However, as previously stated, this effect has been partially corrected by the Trisec® software and is not observed for polymer standards (Fig. 4 and Supporting Information). This is generally the case with modern high resolution columns [28].

The theory of determination of MW by triple detection has been described previously [38,49,60]. This determination involves calculating the form factor iteratively using the following equation to approximate the radius of gyration, R_g:[3,60]

$$R_g = \frac{1}{\sqrt{6}}\left(\frac{[\eta]M}{\Phi}\right)^{1/3} \qquad (2)$$

where Φ is the Flory-Fox coefficient. The local polydispersity leads an underestimation of the form factor calculated by triple detection [49]. The MW determined by triple detection is thus an overestimate of the expected $\overline{M}_w(V_h)$. However, it has been shown that polydispersity has a little influence on the value of the form factor [61] compared to the difference observed between $\overline{M}_n(V_h)$ and $\overline{M}_w(V_h)$ observed on a wide range of V_h. The measured local polydispersity index could also be overestimated because of the increase up to a factor 2 of the Flory-Fox coefficient with the branching and MW of the polymers [3,62]. Note however that the Flory-Fox coefficient is not used here for universal calibration, but is used to calculate MW by the triple detection method. However, it is used for higher MW (lower t_{el}), when the form factor becomes less than unity and RALLS (right-angle laser light scattering) alone can no longer be used for MW determination. RALLS consists of determining the MW using the Rayleigh equation together with the single 90° angle assuming a value of unity for the form factor. If the value of the Flory-Fox coefficient had a

significant negative impact on the MW determined by triple detection, then triple detection and universal calibration would agree at lower MWs (high t_{el}) and differ at higher MWs, i.e. the measured polydispersity $I_p(V_h)$ would increase with V_h (decrease with t_{el}). However, the contrary is experimentally observed. Second, the Flory-Fox coefficient is not used in the LALLS calculation, and LALLS and triple detection agree within experimental error, giving a second proof that the effect of the value of the Flory-Fox coefficient on the calculation of the local polydispersity is negligible compared to that of the actual local polydispersity of the sample. Note that the values of radius of gyration are not given in this work. They cannot be determined by LALLS or triple detection. Values of radius of gyration can be calculated from the molecular weight values and equation (2); however, these values cannot bring any additional information because of the validity of equation (2) and the value of the Flory-Fox discussed above and because their qualitative behavior against elution time has to the same as MWs.

Separation of ultrahigh molecular weight polymers often suffers from molecular degradation, viscous fingering etc. [63]. However, none of the samples analyzed in this work contain a significant amount of ultrahigh molecular weights. The local polydispersity reach in fact its highest value for the lowest molecular weights (obtained by nitroxide-mediated polymerization). The highest molecular weight standards have a more pronounced tail toward elution time (Supporting Information) indicating some degradation may occur for these standards due to relatively high flowrate and low column particle sizes used in this work.

Complete SEC separation for highly branched polyacrylates

Hamielec *et al.* [25] predicted local polydispersity index to increase above unity at lower t_{el} where the molecular weights are larger and the long-chain branching frequencies are higher, i.e. $I_p(V_h)$ should increase with V_h. Fig. 6 shows the opposite trend in the present system. This can be rationalized because the number of long-chain branches per chain should increase with the chain length, since long-chain branches are likely to originate from intermolecular chain transfer to polymer [64,65] and the probability of this transfer reaction scales with the number of monomer unit in the polymer chain, i.e with its molecular weight. Different topologies are thus obtained at different V_h. At a given V_h, the difference in MW between a linear chain, a 3-arm star, a H-shaped chain, etc. should be significant. When the number of branches per chain, n, is higher, i.e. for the longer chains, the difference of MW between chains of the same V_h with n and n+1 long-chain branches should become smaller [66]. This may explain why $I_p(V_h)$ decreases with increasing V_h even if the number of long-chain branches per chain increases with increasing V_h. Further investigation is needed to confirm this explanation. While the influence of regular branching in SEC is well understood and has been successfully modeled [67], the situation of complex branched polymers is far less understood and far more complex because of the change of the branching topology and distribution of length of the branches with V_h. Note $I_p(V_h)$ increase when the branching level decrease as long as some chains are branched; in the case of linear (or regular-branched) homopolymers, there is a one-to-one relation between MW and V_h and thus $I_p(V_h)$ is unity, i.e. the separation is complete in terms of MW (assuming no band-broadening). If one could plot $I_p(V_h)$ against average branching level for a given type of polymer on a given experimental set-up, a discontinuity should thus been observed.

The mechanism of separation is important for the detection of long-chain branching. The separation of complex branched polymers by SEC is indeed more sensitive to a small average number of long-chain branches per chain (leading to a mixture of significantly different topologies) than to a high one (leading to similar topologies). Thus, the averaged local polydispersity index is higher for slightly branched polyacrylate produced by PLP at low conversion or nitroxide-mediated controlled free-radical polymerization at high conversion (Fig. 6 b) than for a highly-branched

polyacrylate produced by conventional free-radical polymerization at high conversion (Fig. 6a). While nitroxide-mediated controlled free-radical polymerization generally yields polyacrylates with significantly less than one long-chain branch per chain (Fig. 4a and reference [53]), targeting high MW (1.07×10^5 g·mol^{-1}) leads to more branches per chain and then high local polydispersity index up to 2.5 (Fig. 5b). The local polydispersity index is lower, below 1.5 when a polyacrylate which may contain more long-chain branches per chain is measured, since it is obtained by conventional free-radical polymerization. This is illustrated by Fig. 6a for poly(ethyl acrylate). The same range of $I_p(V_h)$ is obtained for poly(methyl acrylate) obtained in the same conditions (data not shown [42]). Increasing the long-chain branching level even more should make it thus possible to obtain a very low and negligible local polydispersity index $I_p(V_h)$: on Figure 6, $I_p(V_h)$ reach values very close to unity at highest V_h, i.e. highest long-chain branching levels for polyacrylates.

This is also consistent with the Mark-Houwink plots observed with low density polyethylene (LDPE) [13] or branched polystyrenes [3], as follows. The mechanism of formation of the branches in LDPE (free-radical polymerization) is the same as in polyacrylates: intermolecular chain transfer to polymer leads to long-chain branching and the probability of long-chain branching increases with the chain length. The longer the LDPE or polyacrylate chains, the higher the long-chain branching level. In the case of LDPE, this has been extensively proven to lead to a curved Mark-Houwink plot [13], with the intrinsic viscosity leveling off at high chain length/branching level. This is consistent with our observation: for sufficiently highly branched polymers, the intrinsic viscosity does not vary with MW (see the case of hyperbranched polymers [68]) and there is then a one-to-one relation between V_h and MW. This one-to-one relation implies complete separation of the species by SEC and thus a local polydispersity index of one as measured by Mueller *et al.* for highly-branched poly(methyl methacrylate) [21]. Jackson *et al.* [27] may have produced such samples using methyl methacrylate and a difunctional comonomer. They observed that the $\overline{M}_w$s for the whole distribution obtained from offline static light scattering and SEC-MALLS are in good agreement. Gnanou *et al.* [69] observed the same agreement between offline static light scattering and SEC-MALLS for 7th generation dendrimer-like, and not dendrimers, poly(ethylene oxide). Since offline static light scattering is not affected by local polydispersity problems, one can conclude that for these samples with higher long-chain branching level, the local polydispersity is low and negligible. Recent Monte Carlo simulations of hyperbranched polymers [70] confirm that the local polydispersity index should be very close to unity for this type of randomly hyperbranched polymer [71]. This confirms the validity of the SEC MALLS analysis of glycogen, an hyperbranched polymer [6]. The literature on Mark-Houwink-Sakurada parameters of polyacrylates has been reviewed,[40] but Mark-Houwink plots are not informative for the polyacrylates treated here[42]. The molecular weight range may be too narrow and Mark-Houwink plots exhibit noise on both axis, log$M$ and log[η], and in the case of polyacrylates, low dn/dc values make this noise too important.

The same situation might apply for the separation of the two main components of starch: amylose and amylopectin, as long as universal calibration holds. Since amylopectin is highly branched while amylose is slightly branched [72], the averaged local polydispersity index may be higher for amylose than for amylopectin. One has however to be careful when dealing with very high branching levels, as found in amylopectin or hyperbranched polymers in general. This is for at least two reasons. First, the increase in the number of end-groups (chemically different from the monomeric unit) with respect to the number of monomeric units might favor their interactions with the stationary phase. This would change the mechanism of the separation by SEC and lead to the non-validity of universal calibration when the end-groups exhibit significant enthalpic interactions. This depends on both the number and nature of the end-groups [12,22]. Second, the equations used

to calculate the V_h and the form factor expression for triple detection or MALLS have been derived for linear chains and not for complex branched polymers [49].

Conclusions

Complex branched polyacrylates have been separated by SEC. Multiple-detection SEC enables to determine molecular weight by two independent methods: viscometry (relying on universal calibration) and light scattering, and thus validate or not the size-exclusion mechanism of separation. While multiple-detection SEC is a unique tool to detect even very low level of long-chain branches, the use of multiple-detectors does not guarantee the determination of true molecular weights and critical comparison of universal calibration and light scattering results is thus highly recommended for complex polymers. Complete separation in SEC (in terms of molecular weight) can be obtained for highly branched polymers if interactions with the stationary phase are minimized. A true size-exclusion separation takes place for poly(alkyl acrylates) in THF but the presence of a low level of long-chain branches prevents a complete separation in terms of molecular weight.

The treatment of data obtained from the SEC separation of statistically and slightly long-chain branched polyacrylates as well as the determination of the MW by multiple-detection SEC techniques has been carefully examined. Multiple-detection SEC yields noisy raw molecular weight distribution (MWD) and can significantly overestimate molecular weight at high t_{el} (a phenomenon known as "anomalous elution" although it is most probably a data treatment problem rather than a separation mechanism one). To overcome this, it is recommended to co-add and fit the data: collecting all the log$M$ against t_{el} for samples having the same branching distribution. This makes it possible to accurately compare the MWs of slightly branched polyacrylates obtained by three different techniques on the same sample: universal calibration, triple detection and LALLS. Triple detection and LALLS appear to yield systematically higher MWs than universal calibration. This is attributed to the presence of a distribution of branching topologies, which induces an incomplete separation according to MW in SEC. Due to the well-known phenomenon that SEC separates by size and not MW, a slice of statistically branched polymer eluting at a given elution time will indeed have a distribution of MWs. The true molecular weight distribution (MWD) cannot be determined by SEC in that case, even using multiple-detection. At each elution time, an average MW is determined: $\overline{M}_n(V_h)$ for viscometry (relying on universal calibration) and $\overline{M}_w(V_h)$ for light scattering. However, more information can be derived using multiple detection as shown theoretically [49]. The local polydispersity and averaged local polydispersity indices decrease when long-chain branching level increases. For highly branched polymers, the SEC separation is complete in terms of molecular weight and the true molecular weight distribution can be determined.

When true molecular weight can not be determined by SEC, comparative studies can still be undertaken using hydrodynamic volume distributions,[49] as recently illustrated in the study of controlled/living polymerization[73] or the mechanism of action of starch branching enzymes[74]. Furthermore, the number and weight average molecular weight of the whole MWD, $\overline{M}_n$ and $\overline{M}_w$ can be determined from the hydrodynamic volume distributions and respectively the local $\overline{M}_n(V_h)$ and $\overline{M}_w(V_h)$ even if the MWD itself can not be determined [49]. Complete separation of slightly branched polyacrylates requires two-dimensional liquid chromatography methods [75] and regularly-branched polymers have been already successfully separated by this method (e.g. [76,77]). Separating complex branched polymers is a major challenge for polymer separation science.

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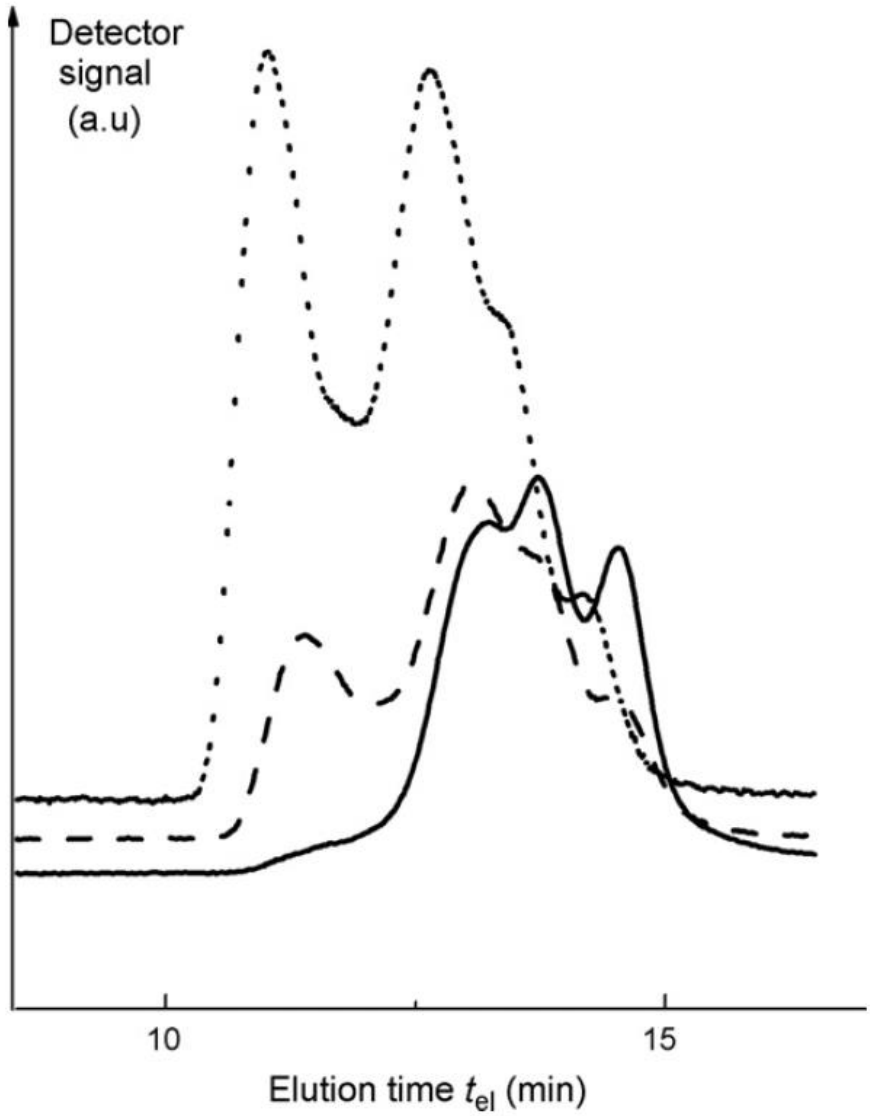
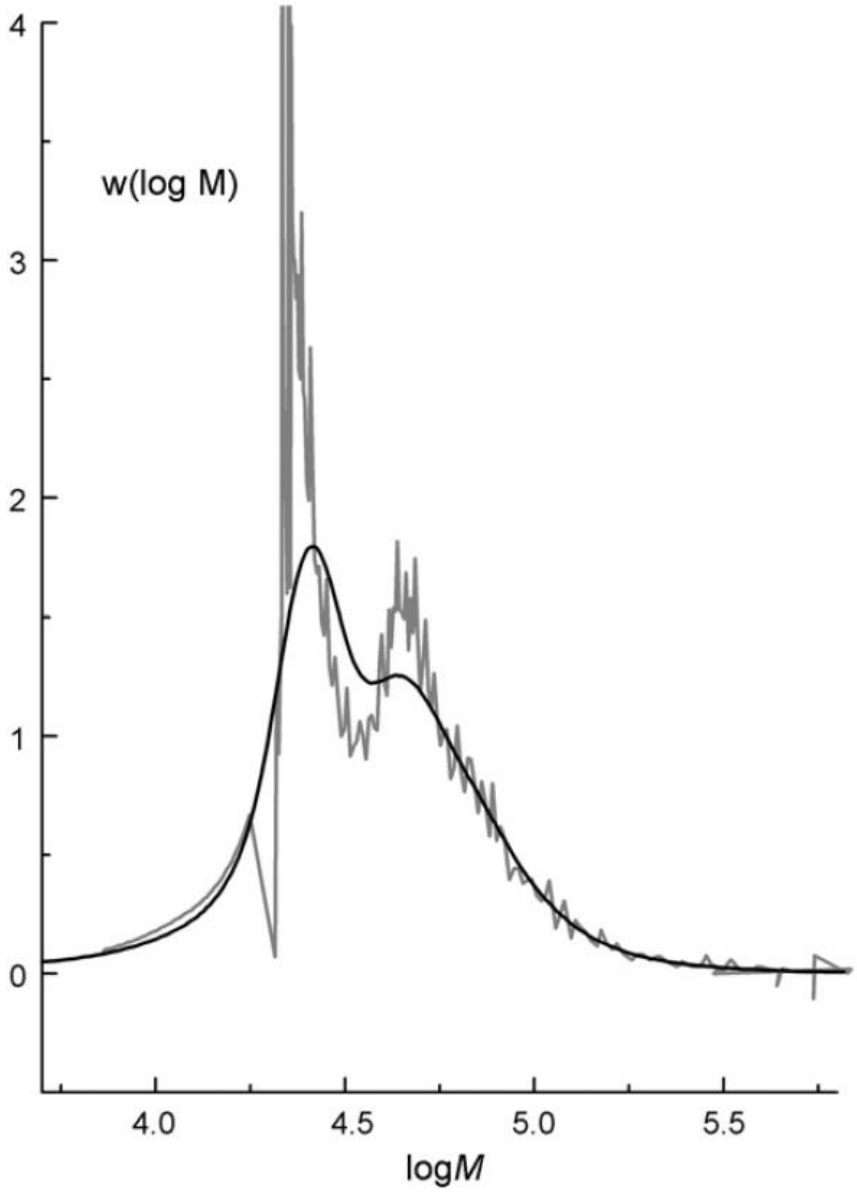
606 ***Figures***



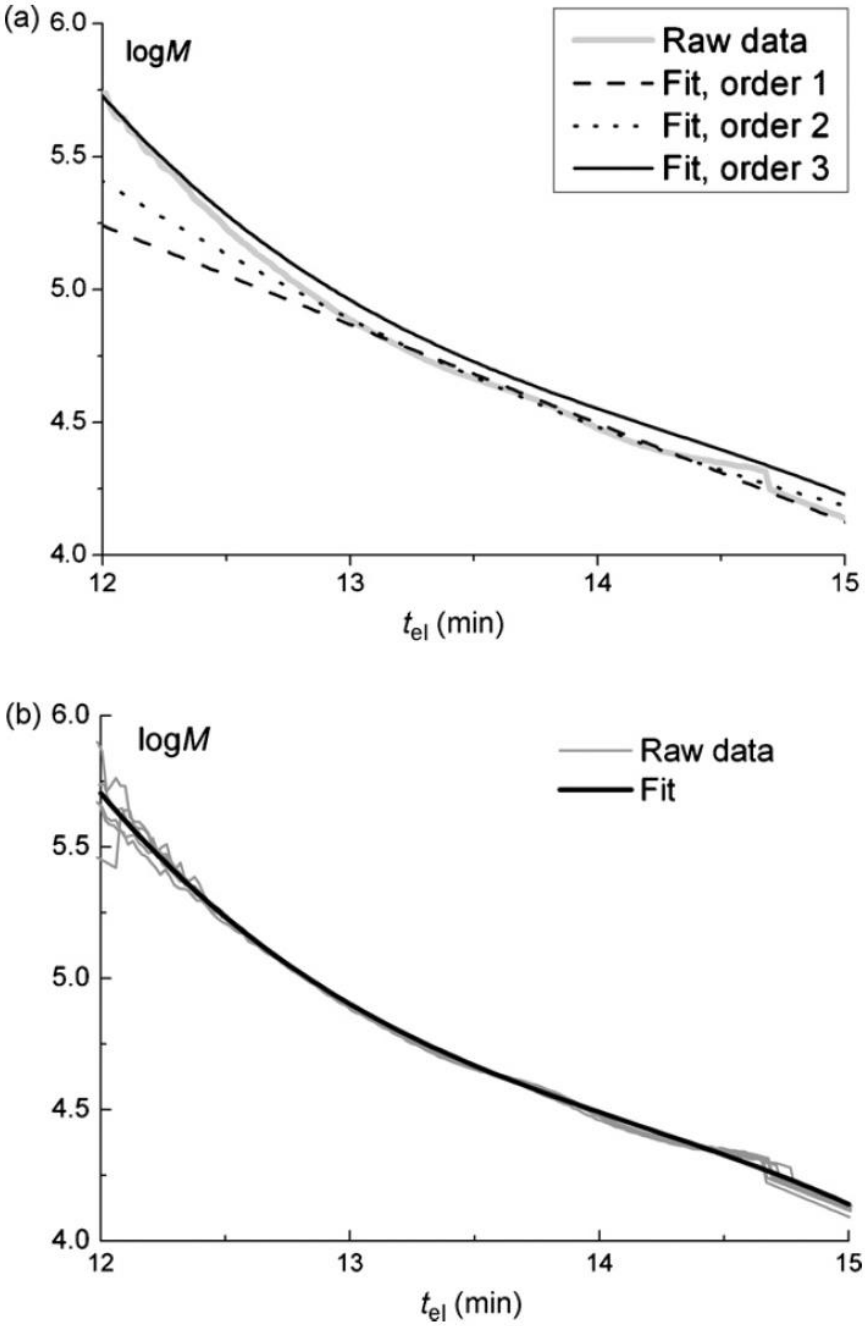
607

608 **Figure 1.** Triple-detector SEC chromatograms (raw data) of a poly(2-ethylhexyl acrylate) obtained
609 by PLP at –5°C, 100 Hz: refractometer (solid line), viscometer (dashed line), RALLS (dotted line).



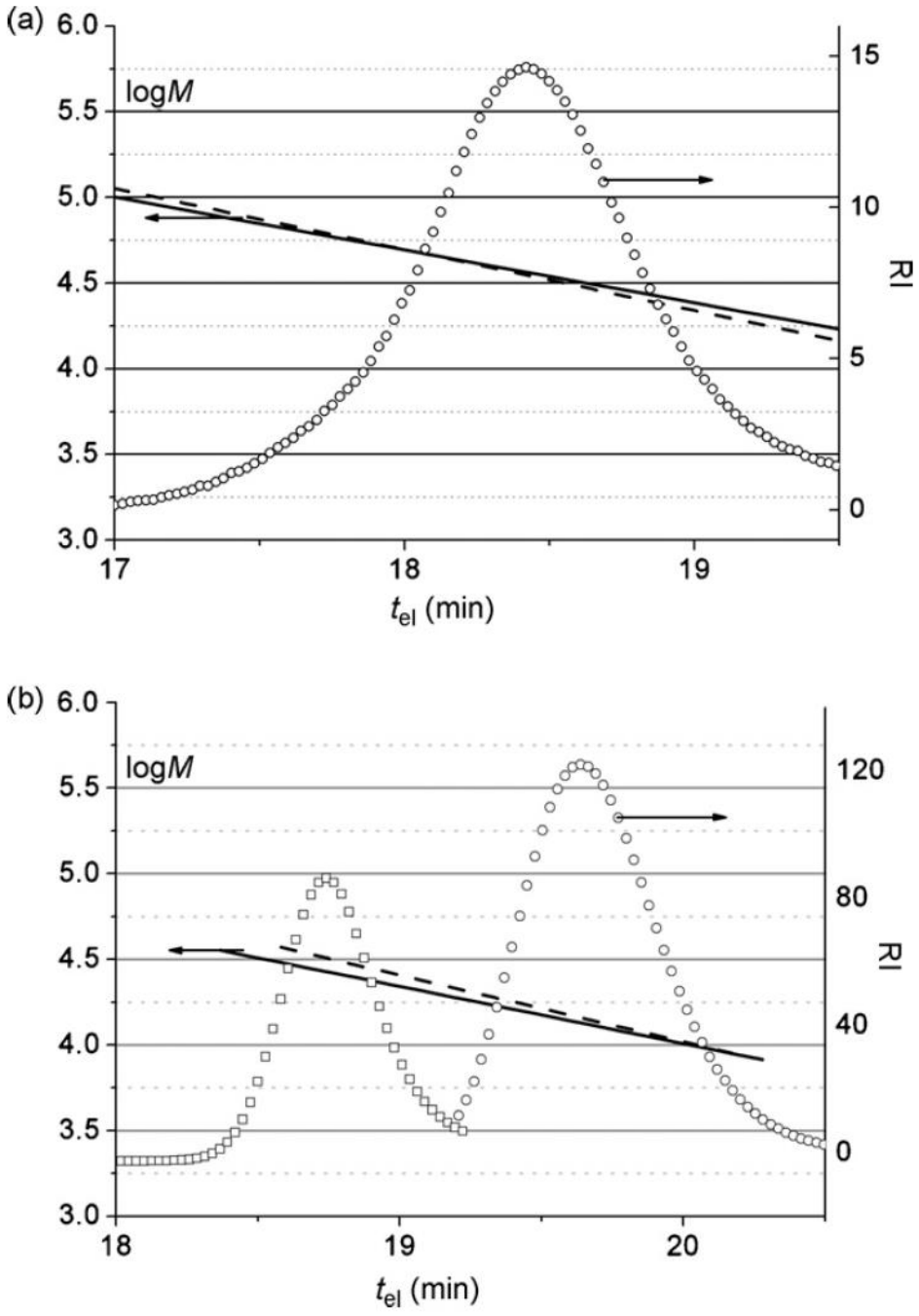
610

611 **Figure 2.** MWD obtained by Triple Detection SEC treatment (Trisec®) (gray line) of the
612 chromatograms of Fig. 1 and MWD obtained after fitting (black line) these data on the log*M* against
613 t_{el}.



614

615 **Figure 3.** Fits of the log$M$ against t_{el} by a polynomial (1st, 2nd and 3rd order), obtained by triple
616 detection using Trisec® software for a single injection (top) and by fitting (3rd order) several
617 injections of poly(2-ethylhexyl acrylate) obtained at the same temperature and monomer
618 concentration (bottom).



619

620 **Figure 4.** Comparison of the fit of log*M* against t_{el} obtained by independent methods: universal
621 calibration (dashed line) and triple detection (solid line) for some poly(*n*-butyl acrylate) samples
622 obtained by two different types of polymerization: nitroxide-mediated free-radical polymerization
623 targeting low MW (a), anionic polymerization (two samples) (b). The refractometer signal is
624 displayed with open symbols.

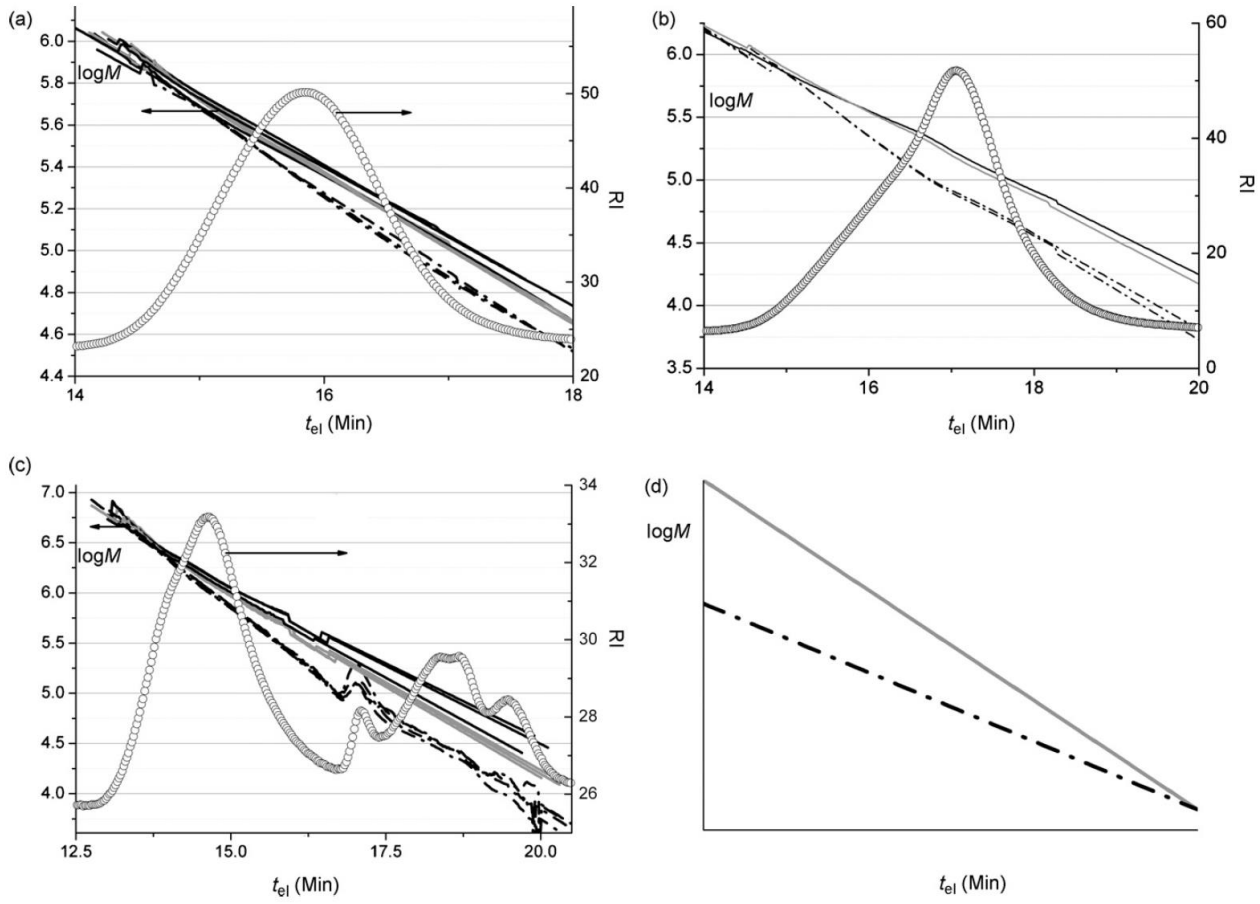
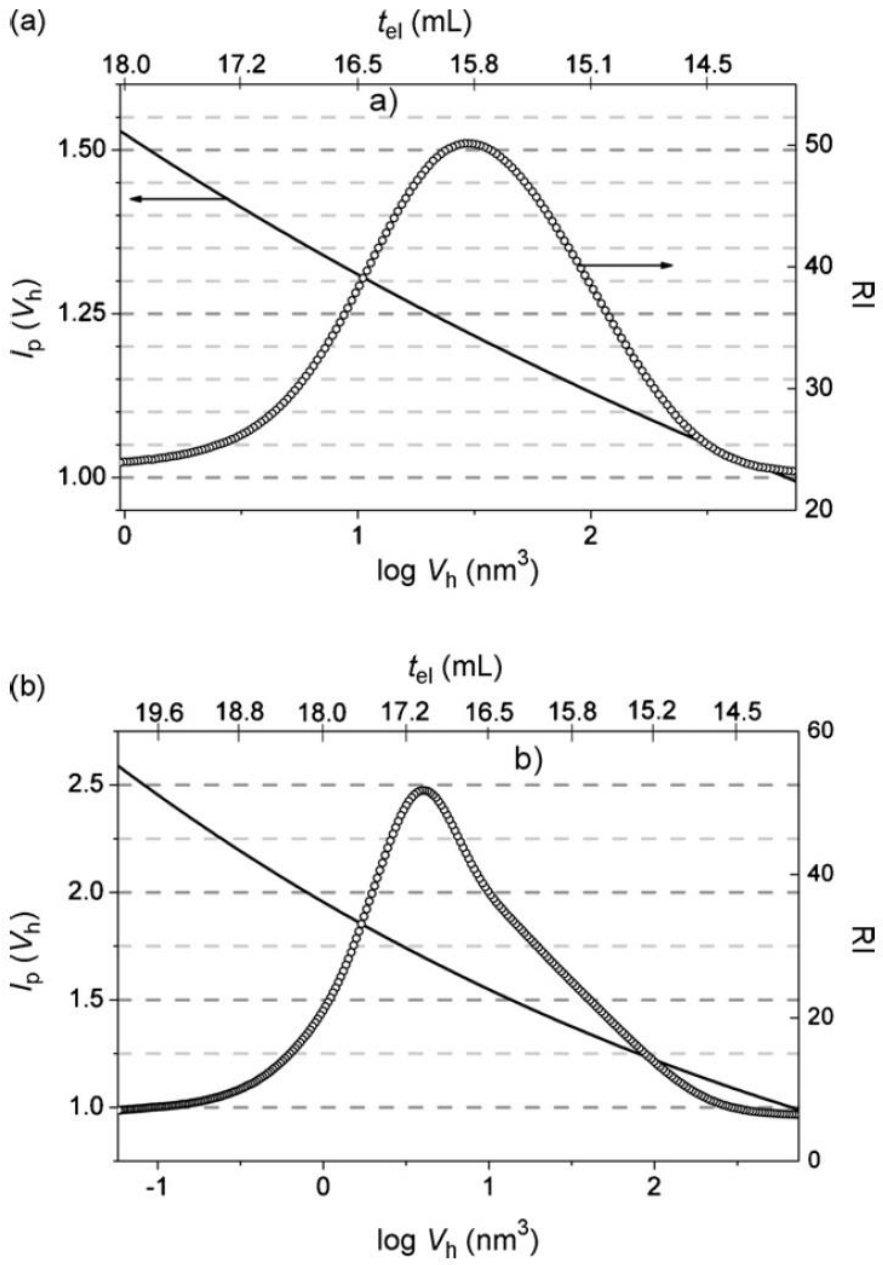


Figure 5. Comparison of the raw log*M* against t_{el} obtained by different methods: universal calibration (dashed dotted line), triple detection (solid gray line) and low angle laser light scattering (LALLS, solid black line) for some long-chain branched polyacrylates obtained by three different types of polymerization: conventional free-radical polymerization (poly(ethyl acrylate)) at high conversion and injected at 9.8 $g \cdot L^{-1}$ (a), nitroxide-mediated free-radical polymerization (poly(*n*-butyl acrylate) at high conversion and targeting high MW) (b), pulsed-laser polymerization (poly(2-ethylhexyl acrylate)) at low conversion (c); prediction by Balke *et al.* [26] for polymers obtained by polycondensation (d). The refractometer signal is displayed with open circles.



634

635 **Figure 6.** Variation of the local polydispersity index, $I_p(V_h)$, with hydrodynamic volume V_h for
636 some polyacrylates obtained by different types of polymerization: conventional free-radical
637 polymerization (poly(ethyl acrylate), Fig. 5a) (a), nitroxide-mediated free-radical polymerization
638 (poly(*n*-butyl acrylate), Fig. 5b) targeting high MW (b). Elution time, t_{el}, has been added as top x
639 axis to allow comparison with figure 5; note that the scale is then not linear (a third-oder
640 polynomial relation between log V_h and t_{el} has been used).

Supporting Information

Multiple-detection SEC of complex branched polyacrylates

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Polymerizations

Pulsed-Laser Polymerization of 2-ethylhexyl acrylate

2-ethylhexyl (2EHA) acrylate (purity ≥ 99.5 %, stabilized by 20 ppm of 4-methoxyphenol) was provided by Arkema, distilled under reduced pressure and systematically injected into the SEC to check the absence of preformed polyacrylates prior to use. Monomer solutions in toluene (50 wt%), containing 5×10^{-3} mol·L^{-1} of photoinitiator (2,2-dimethoxy-2-phenylacetophenone-DMPA) (Aldrich), were carefully deoxygenated by flushing the cell with nitrogen before being submitted to laser pulses to perform photopolymerization. The photopolymerization process and laser set-up have been described previously.[1] The remaining monomer and the solvent were evaporated at room temperature and atmospheric pressure in aluminum pans before SEC analysis.

Conventional free-radical polymerization of ethyl acrylate

Ethyl acrylate (Aldrich, 99 %, stabilized with 15-20 ppm monomethyl ether hydroquinone) was distilled under reduced pressure and stored at -20 °C. Ethyl acrylate was polymerized by initiating with 0.5 mol% of AIBN with respect to the acrylic monomer. The polymerization was carried out at 60 °C under nitrogen for 20 h. The resulting reaction mixture was dissolved in an equal volume of dichloromethane. The polymer was then precipitated in methanol over liquid nitrogen at a temperature lower than its glass transition temperature (T_g), filtered and finally dried in an oven (60 °C) under vacuum for one night. The glass transition temperature of the polymer was measured to be –14 °C by DSC at 10 °C·min^{-1}. [2] ^{13}C NMR measurements determined that it was atactic [2].

Nitroxide-mediated free-radical polymerization of n-butyl acrylate

n-Butyl acrylate (nBA, Aldrich, 99 %) was distilled under reduced pressure before use. Dowfax 8930 and the buffer, sodium hydrogen carbonate ($NaHCO_3$, Prolabo, >99 %) were used as received. Hexadecane (Aldrich, >99 %) and high molecular weight polystyrene (Arkema, $\overline{M}_w$ = 330,000 g·mol^{-1}) were also used as received. The SG1 nitroxide (86 %), the SG1-based oil-soluble dialkoxyamine, DIAMS (90 %) and the SG1-based alkoxyamine derived from methacrylic acid, MAMA (also called BlocBuilder^{TM}, 99%), were kindly supplied by Arkema (their chemical formula are given in Figure 1).

High conversion poly(*n*-butyl acrylate) was obtained by miniemulsion polymerization, using the the following method targeting high MW. A stable aqueous emulsion of nBA was prepared by mixing the organic phase with the water phase containing 406.9 g of deionized water, 2.24 g of Dowfax8390 (2.2 wt% with respect to nBA) and 0.429 g of $NaHCO_3$ (5.11 mmol, 12 mmol.L$^{-1}_{water}$). The organic phase contained 99.57 g of nBA (0.778 mol), 0.753 g of DIAMS (0.92 mmol), 0.0076 g of free SG1 (0.026 mmol, 2.4 mol.% with respect to the alkoxyamine), 0.114 g (0.1 wt% with respect to nBA) of high MW polystyrene, 0.782 g of hexadecane (8.94 mmol, 0.8 wt% with respect to nBA). Polystyrene and hexadecane were used as hydrophobes to stabilize the monomer droplets against Ostwald ripening. The unstable emulsion formed when these components are mixed was then subjected to ultrasonication

(Branson 450 Sonifier; power 7; 10 min) in order to disperse the organic phase into submicronic droplets. This led to a stable miniemulsion, which was deoxygenated by bubbling nitrogen for 20 min at room temperature and then poured into a 600 mL glass thermostated reactor heated at 112 °C (time zero of the reaction) and stirred at 300 rpm. A 3 bar pressure of nitrogen was then applied. After 8 h of polymerization, the reactor was cooled in an iced water bath. The final latex was dried in a ventilated oven thermostated at 70 °C until constant weight. The conversion was 88 %, whence the theoretical $\overline{M}_n$ at this conversion was 107,000 g·mol^{-1}.

Another poly(*n*-butyl acrylate) sample was obtained by bulk polymerization, using the following method targeting low MW. A mixture of the MAMA alkoxyamine (1.894 g, 4.97 mmol) and *n*-butyl acrylate (162.9 g, 1.27 mmol) was deoxygenated with nitrogen bubbling for 20 min at room temperature. The mixture was poured into a 300 mL thermostated glass reactor heated at 112 °C and stirred at 300 rpm. A 1 bar pressure of nitrogen was then applied to allow sample withdrawal. The samples were dried to measure the conversion, 80 %, by gravimetry in a ventilated oven thermostated at 70 °C until constant weight. The theoretical $\overline{M}_n$ at this conversion was 25,200 g·mol^{-1}.

Figure 1. Chemical formula of some chemicals used in the nitroxide-mediated controlled free radical polymerization:

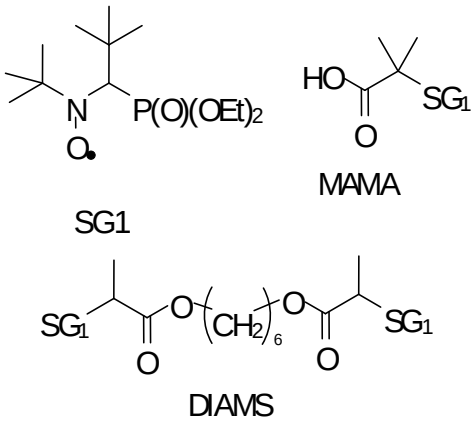
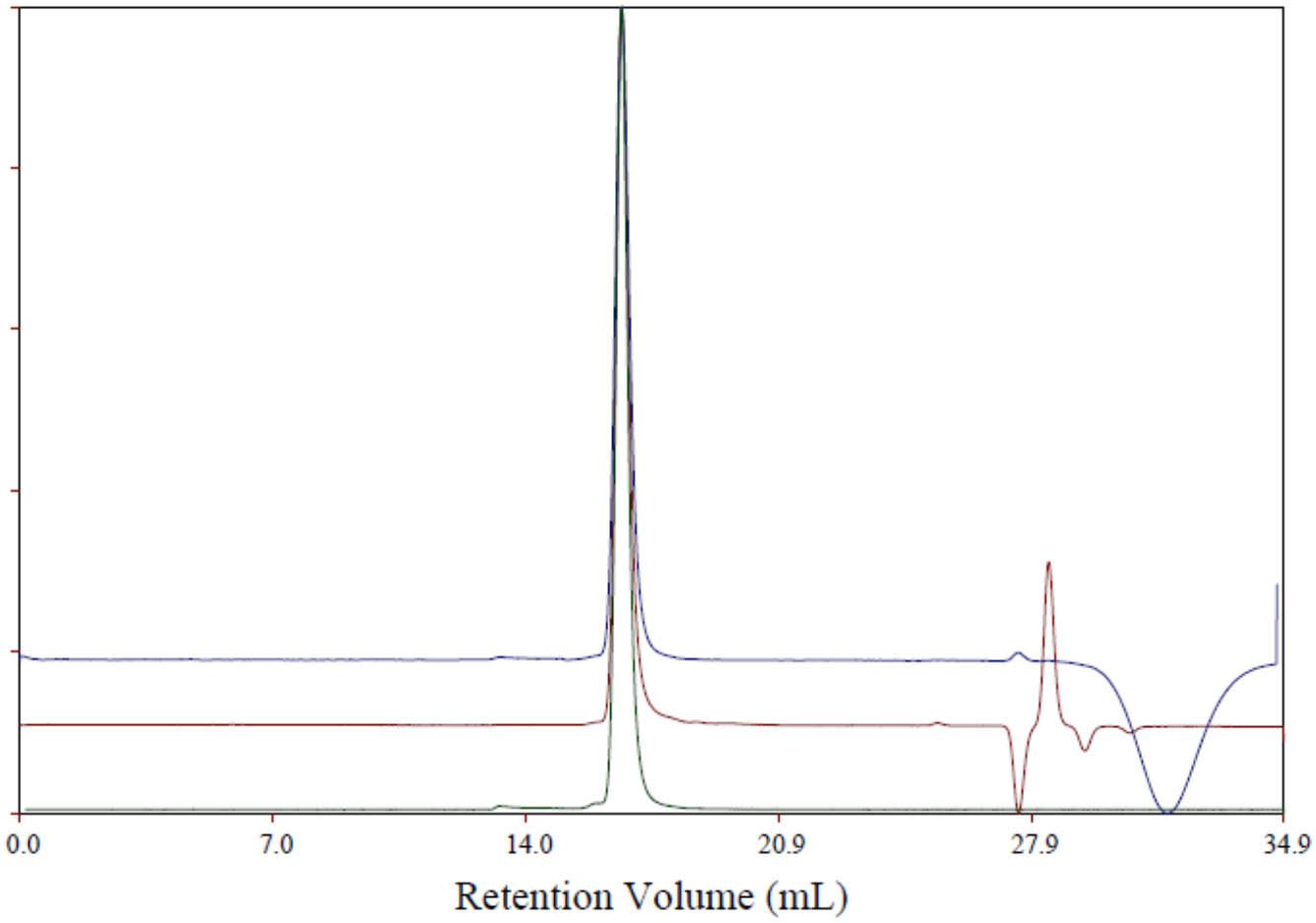
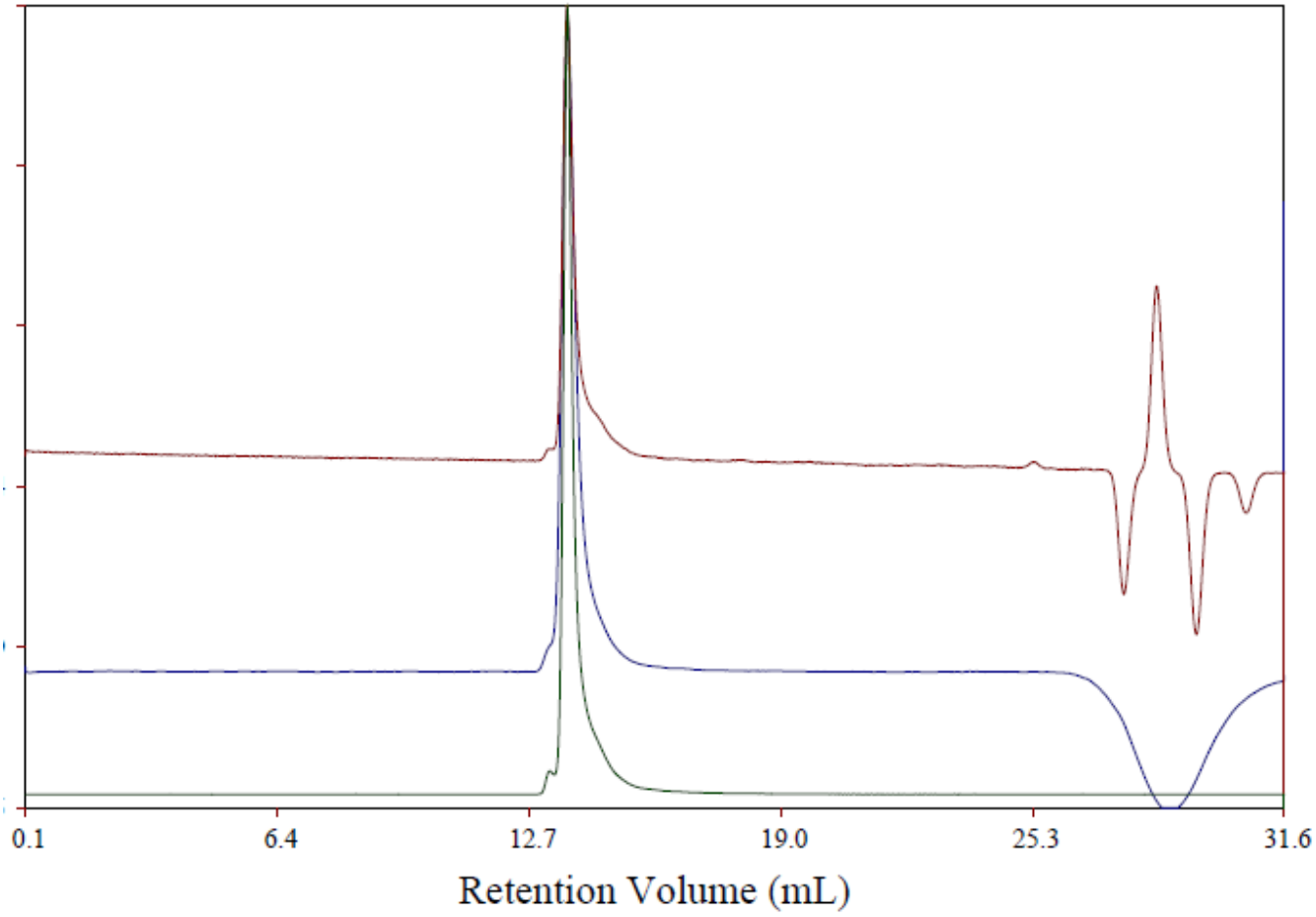


Figure 2. Example of chromatograms for polystyrene standards:

- PSS standards, peak molecular weight M_p = 67,500, injected during period 1 (see next tables). From top to bottom at the y intercept, signals correspond to viscometer, refractometer, right-angle laser light scattering.



- PSS standards, M_p = 2.570.000, injected during period 1 (see next tables). From top to bottom at the y intercept, signals correspond to refractometer, viscometer, right-angle laser light scattering



- PSS standard, oligomers (see * at bottom of Figure 3), injected during period 2 (see next tables). Only the refractometer trace is displayed (viscometer and light scattering are highly noisy signals because of low molecular weight). Complete characterization of this mixture of oligomers has been achieved by 2D HPLC [3].

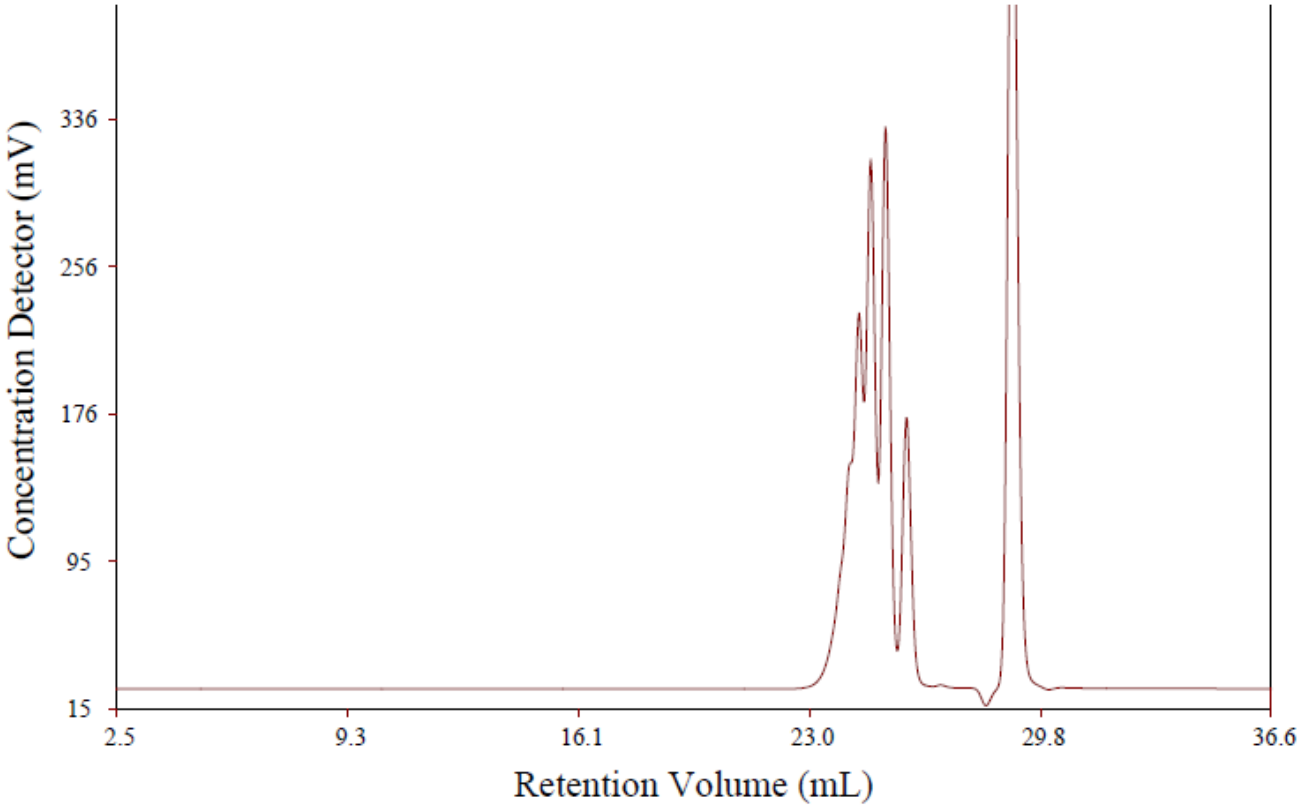
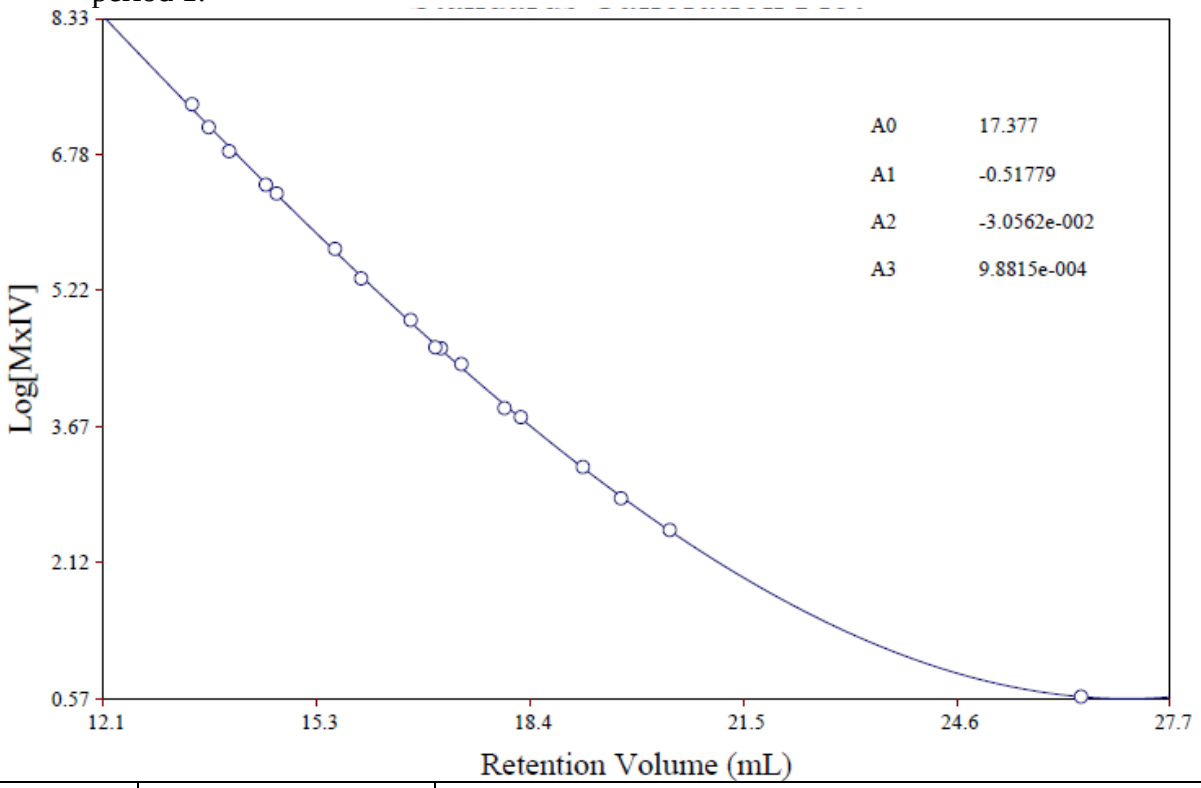


Figure 3. Universal calibration curves obtained at different times of use of the SEC equipment related to this work. IV stands for intrinsic viscosity and A0 to A3 are the fit of the curve by a polynomial function $\log [\eta]M = A0 + A1.Vel + A2.Vel^2 + A3.Vel^3$.where Vel is the elution volume and $[\eta]M$ is in $dL \cdot mol^{-1}$.

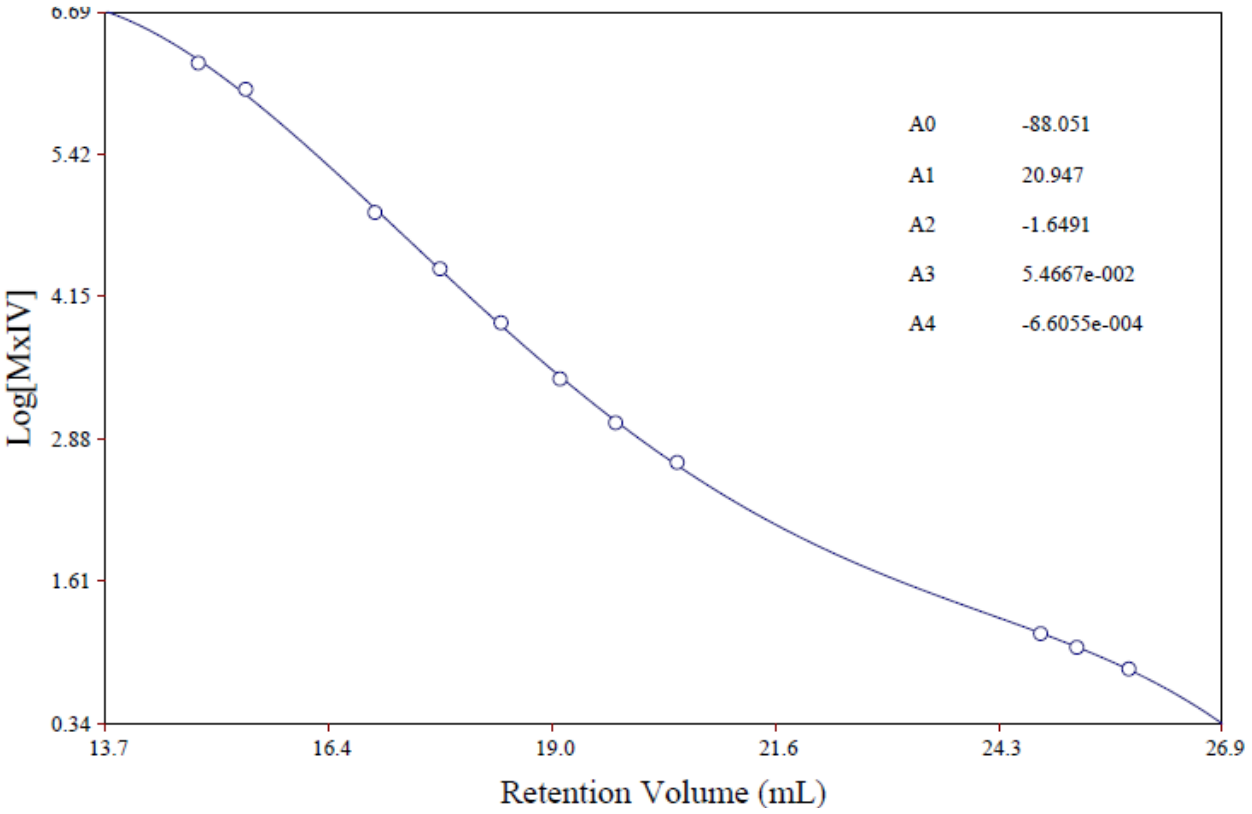
- period 1:



*M*p	Elution volume (mL)	Deviation between the fit of the calibration curve and the input M_p (%)
162	26.40	+0.1
4,920	20.40	+1.9
8,390	19.69	-4.9
13,400	19.14	+2.1
29,600	18.23	+2.9
34,800	18.00	-8.7
67,500	17.37	+9.8
89,300	16.99	-7.0

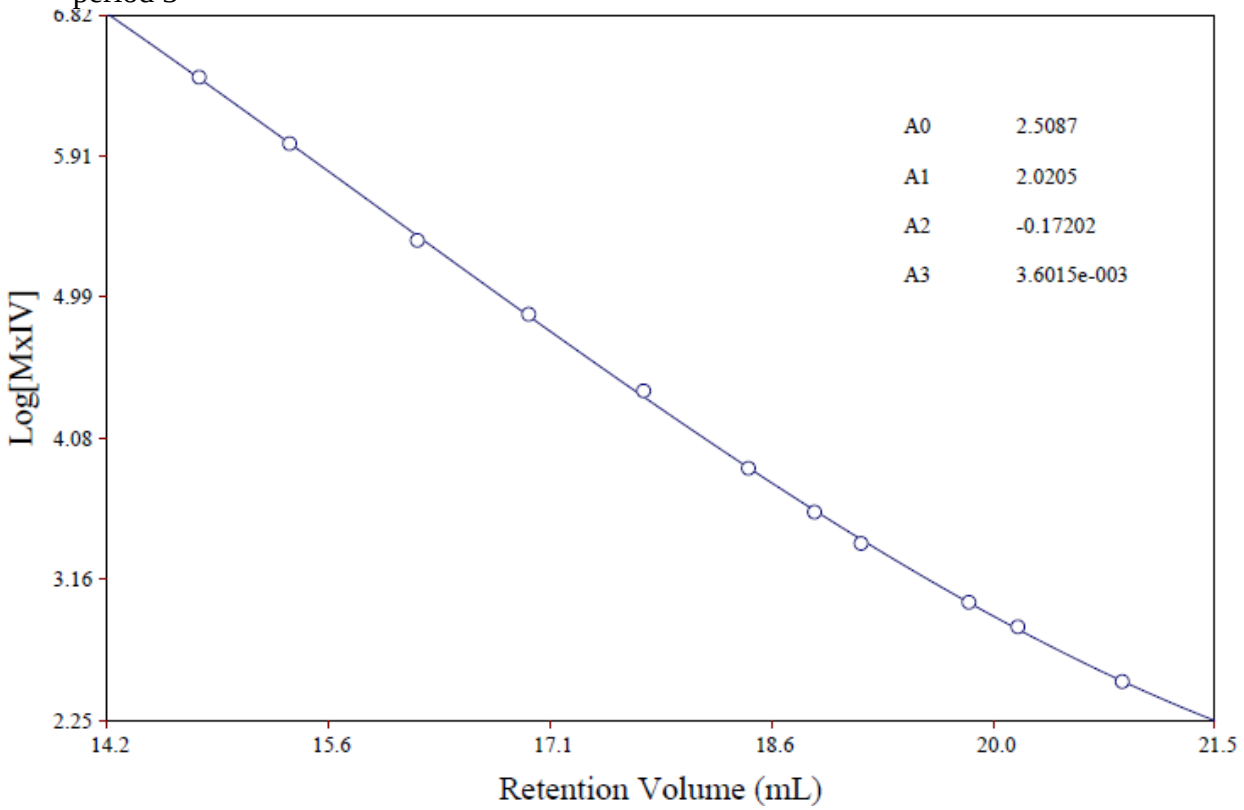
89,300	17.07	+3.6
130,000	16.63	+6.1
246,000	15.90	-5.7
400,000	15.52	+6.5
940,000	14.67	+2.2
1,090,000	14.52	-3.0
1,870,000	13.98	-11.4
2,570,000	13.68	-3.5
3,800,000	13.44	+12.6

- period 2



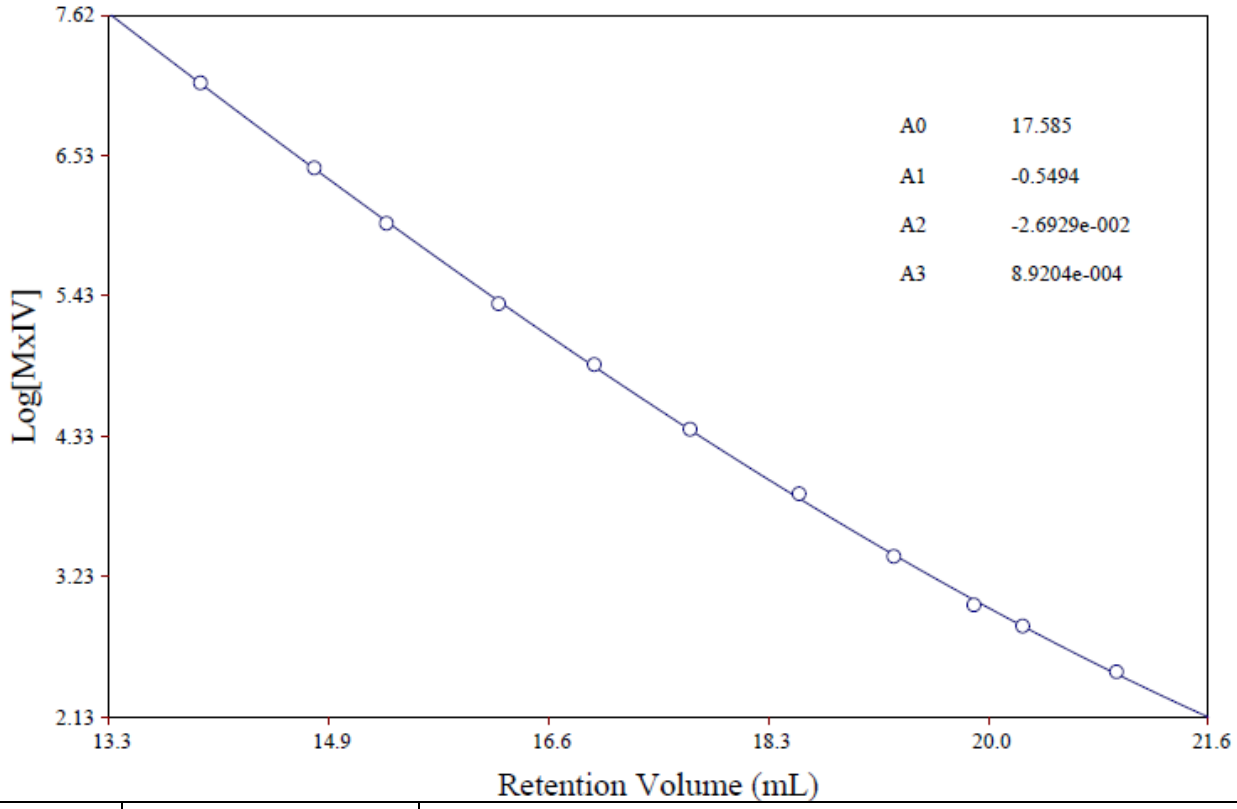
*M*p	Elution volume (mL)	Deviation between the fit of the calibration curve and the input M_p (%)
266*	25.81	+1.3
370*	25.20	-0.8
474*	24.77	-1.3
6,040	20.47	+6.1
10,400	19.75	-3.6
18,100	19.09	-4.9
34,800	18.39	+5.5
67,500	17.67	+1.5
130,000	16.90	-7.7
579,000	15.38	+11.9
1,090,000	14.81	-6.3

- period 3



*M*p	Elution volume (mL)	Deviation between the fit of the calibration curve and the input M_p (%)
4,920	20.84	-0.7
8,390	20.16	+3.7
10,400	19.84	+0.6
18,100	19.13	-5.9
24,000	18.83	-2.5
34,800	18.39	-0.9
67,500	17.71	+9.4
130,000	16.95	+2.6
246,000	16.22	-6.6
579,000	15.39	-0.5
1,090,000	14.79	+1.7

- period 4



*M*p	Elution volume (mL)	Deviation between the fit of the calibration curve and the input M_p (%)
4,920	20.93	+3.9
8,390	20.22	+0.3
10,400	19.85	-8.6
18,100	19.24	-2.8
34,800	18.52	+8.8
67,500	17.69	+1.2
130,000	16.96	+4.0
246,000	16.23	-4.7
579,000	15.38	-3.5
1,090,000	14.83	+1.4
2,570,000	13.97	+1.2

* The standards whose M_p correspond in fact to a mixture of oligostyrene with butyl and hydrogen as end-groups. The M_p 266, 370, 474, 578 correspond respectively to degrees of polymerization of 2, 3, 4 and 5.

Figure 4: Comparison of the universal calibration curves obtained at different periods

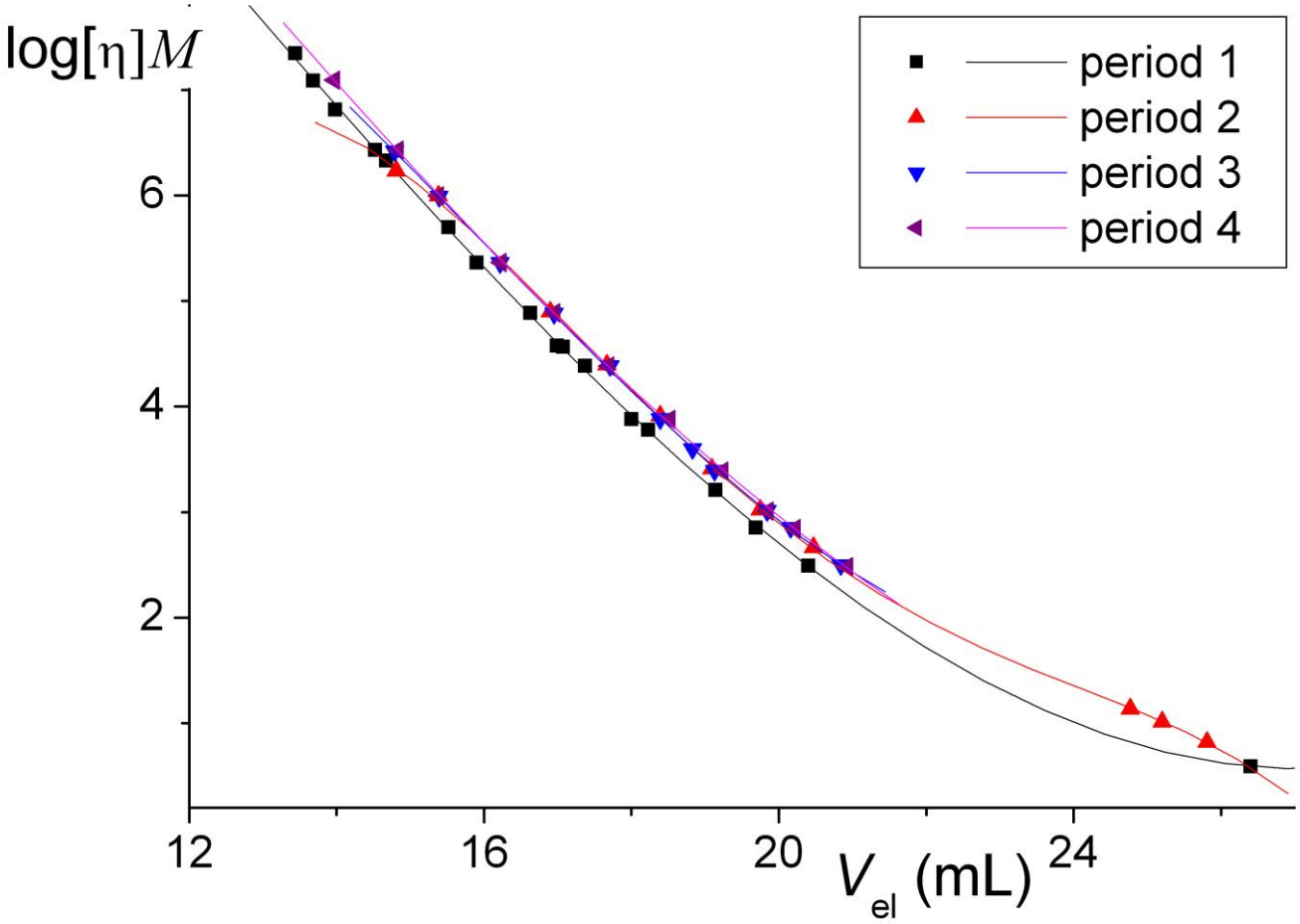


Table 1. Characteristics of the polystyrene standards (PS, PMMA) used to calibrate the multiple detection SEC with the number of time they were used (three calibrations were performed). The final constants were calculated as the arithmetic means of the reasonable values obtained for each standard. Low MWs were not considered for calculation of the average for the calibration of the viscometer and light scattering, while high MWs, i.e. low injection concentrations, were rejected in the case of the refractometer calibration.

Supplier	*M_p ($g \cdot mol^{-1}$) (from supplier)*	*Polydispersity (from supplier)*	*Used to calibrate(the number corresponingd to the number of times this standard has been used for calibration)*		
			Refracto-meter	*Visco-meter*	*Light scattering*
PSS	1,620	1.06	1		
PSS	4,920	1.08	2	1	
PSS	8,390	1.04	2	1	
PSS	10,400	1.03	2	2	2
PSS	18,100	1.03			1
PSS	34,800	1.04		1	2
PSS	67,500	1.02	2	2	4
PSS	130,000	1.04	1		6
PSS	246,000	1.06	1		3
PSS	339,000	1.03	1		1
PSS	579,000	1.03	2	1	2
PSS	1,090,000	1.06	1	2	2
PSS	2,570,000	1.04			2
Viscotek	6,040	1.06			
Viscotek	13,400	1.05	1	1	1
Viscotek	29,600	1.03	1	1	1
Viscotek	64,500	1.02			1
Viscotek	89,300	1.04		1	2
Viscotek	170,000	1.04	2	2	2
Viscotek	400,000	1.04	1	1	1
Viscotek	940,000	1.03		1	1
Viscotek	1,870,000	1.07		1	1
Viscotek	3,800,000	1.04		1	1

Table 2. Calibrations constants of the different detectors: the value is as an average on a number of measurements for different standards (injections from different standards were used depending on the detector. Typically, highest molecular weights are not used for the refractometer since they have to be injected at a too low concentration, while lowest molecular weights give too noisy viscometric and light scattering signals). Accuracy is estimated as the ratio of the standard deviation of the measurement to the average constant value. There are two periods (measuring cells for the refractometer and light scattering were changed in-between)

Period	Mass constant (refractometer)			Viscometer constant			Light scattering constant (90°)		
	Value	Accuracy (%)	Number of standards	Value	Accuracy (%)	Number of standards	Value $\times 10^5$	Accuracy (%)	Number of standards
1-3	1824	3.0	8	1090	2.1	12	29.51	2.4	15
4	1162	4.4	8	1063	2.5	8	22.58	5.9	10

Table 3. Peak parameters (skew σ and variance τ for band broadening and offset inter-detector delay corrections) used with Trisec software

Period	Refractometer		Viscometer			Light scattering		
	σ	τ	σ	τ	offset	σ	τ	offset
1-4	0.138	0.121	0.138	0.158	-0.187	0.138	0.094	0.163

Table 4. Benchmark of the triple detector SEC set-up using polystyrene (PS), poly(methyl methacrylate) (PMMA) or poly(*n*-butyl acrylate) (PnBA) standards. After an average period of two months, the set-up was calibrated again following technical problem or just observation that the calibration no longer held. Each period of time given below correspond to one calibration. C is the injection concentration. Diff. is the relative difference between the measured M_p nad the value indicated by the supplier.

- period 1

Polymer (supplier)	*C* $g \cdot L^{-1}$	M_p *(from supplier)*	*universal calibration*		*triple detection*		*LALLS*	
			M_p	*Diff.*	M_p	*Diff.*	M_p	*Diff.*
PS (PSS)	-	18,100	22,000	+19	16,300	-10	15,900	-13
PS (PSS)	5.2	67,500	68,800	+2	69,600	+3	70,900	+5
			66,200	-2	64,200	-5	64,300	-5
			68,200	+1	65,800	-3	64,600	-4
			69,700	+3	64,500	-4,5	63,700	-6
			73,900	+9	62,500	-8	64,300	-4
PS (PSS)	2.1	246,000	272,200	+10	259,800	+5	258,900	+5
PMMA (Interchim)	-	33,700	33,400	-0,1	30,100	-10	29,800	-10

- period 2

Polymer (supplier)	M_p *(from supplier)*	*universal calibration*		*triple detection*		*LALLS*	
		M_p	*Diff.*	M_p	*Diff.*	M_p	*Diff.*
PS (PSS)	34,800	37,700	+7	32,900	-6	33,800	-3
		37,700	+7	32,700	-6	31,100	-11
PS (PSS)	67,500	73,900	+9	62,500	-8	64,300	-4
PMMA (interchim)	33,700	33,800	+0	32,800	-3	30,500	-10

-period 3

Polymer (supplier)	M_p *(from supplier)*	*universal calibration*		*triple detection*	
		M_p	*Diff.*	M_p	*Diff.*
PS (PSS)	67,500	71,800	+6	69,100	+2
		73,300	+8	69,700	+3
		71,400	+6	71,500	+5
		69,400	+3	72,300	+7
	130,000	143,000	+9	118,000	-10
PS (Viscotek)	29,600	34,500	+9	29,400	-1
PnBA (*)	11,700	14,800	+23	11,800	+0
	22,600	30,800	+30	24,300	+7

*: kindly supplied by Dr. Xavier André, University of Bayreuth

- period 4:

Polymer (supplier)	*Concentration injection* $g \cdot L^{-1}$	M_p *(from supplier)*	*universal calibration*		*triple detection*	
			M_p	*Diff.*	M_p	*Diff.*
PS (PSS)	5.2	67,500	68,700	+2	61,500	-9
	-	130,000	133,300	+2	130,000	0
			132,700	+1	129,500	-0
			130,200	+0	122,100	-6
			131,600	+1	127,300	-2
PMMA (Interchim)	-	33,700	37,800	+12	30,700	-9

Table 5. Specific refractive increment, d*n*/d*c*, values

- values used in this work for THF at 40 °C for the SEC detectors

Polymer	Poly(ethyl acrylate), PEA	Poly(*n*-butyl acrylate), PnBA	Poly(2-ethylhexyl acrylate), P2EHA	PS	PMMA
d*n*/d*c* ($mL.g^{-1}$)	0.071	0.063	0.073	0.185	0.09
Uncertainty(%)	5	-	5	-	-

These values are arithmetic means of the reasonable values determined on the SEC set-up (poly(ethyl acrylate and poly(2-ethylhexyl acrylate) or available in the literature (poly(n-butyl acrylate). and given below.

- d*n*/d*c* values for polyacrylates in THF determined in this work or from the literature

Polymer	*dn/dc* $(mL \cdot mg^{-1})$	*T (°C)*	*Number of samples (λ in nm)*	*MW range* $(g.mol^{-1})$	*Determination method*	*Ref.*
PEA	0.061 ± 0.002	25	6 (546)	$\overline{M}_w = 7.10^4$ to 10^6	RDBP*	[4]
	0.070	30	1 (670)		triple-detection SEC	This work
	0.074	30	1 (670)			
	0.072	30	1 (670)			
	0.072	30	1 (670)			
	0.073	30	1 (670)			
	0.070	30	1 (670)			
	0.076	30	1 (670)			
	0.067	30	1 (670)			
	0.075	30	1 (670)			

	0.084	30	1 (670)			
	0.065	40	1 (670)	High conversion by PLP	triple-detection SEC	
	0.067	40	1 (670)			
	0.068	40	1 (670)			
PnBA	0.063 ± 0.002	25	6 (546)	$\overline{M}_w = 3.10^4$ to 10^6	RDBP*	[4]
	0.0615	25				[5]
P2EHA	0.068 ± 0.002	25	6 (546)	$\overline{M}_w = 10^5$ to 10^6	RDBP*	[4]
	0.068		4 (546)	$\overline{M}_w = 10^5$ to 10^6	RDBP*	[6]
	0.070	25	1 (436)	$\overline{M}_w = 2\times10^6$, I_p=1.2	RDBP*	[7]
	0.079	30	1		triple-detection SEC	This work
	0.073	30	1			
	0.076	30	1			
	0.078	30	1			
	0.076	30	1			
	0.073	30	1			
	0.07	30	1			
	0.068	30	1			
	0.074	30	1			
	0.078	30	1			
	0.069	30	1			
PMMA	0.09					[8]
	0.089	30	(488)			[9]
PS	0.185	30-40			Viscotek	

*RDBP is the Brice-Phoenix differential refractomer.

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