



Application possibilities of preparative size exclusion chromatography to analytical problems in polymer science

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Abstract: The application possibilities of preparative size exclusion chromatography for the detailed analysis of polymer analytes are discussed. Using the example of star-shaped polymer architecture, the possibilities of utilizing fractions obtained from preparative size exclusion chromatography and their subsequent off-line characterization with a variety of hyphenated analytic techniques are discussed and compared to one another. It was, for instance, possible to obtain an absolute SEC calibration for the investigated star-shaped polymers that showed very good agreement with theoretical expected values and values obtained by absolute molecular weight determination techniques by analyzing fractions of different molecular weights of this polymer architecture by MALDI-TOFMS in order to obtain the M_p values required for SEC calibrations. Moreover, the star-shaped polymers were investigated by SEC-viscometry in order to obtain their absolute molecular weight. All analytical results are compared to each other and possibilities as well as limitations are discussed.

Introduction

Separation during size exclusion chromatography (SEC) experiments occurs due to the hydrodynamic volume of the analyte and is therefore based on its molecular size rather than its molecular weight. Therefore, if star-shaped polymeric architectures are investigated by SEC, the calculated molecular weight is normally underestimated since usually linear polymer standards are used for calibration [1, 2]. The reason for this behaviour is the fact that the hydrodynamic volume of a star-shaped polymer is smaller than the hydrodynamic volume of a linear analogue with similar molecular weight [3]. $^1\text{H-NMR}$ can therefore be a useful method to calculate the absolute molecular weight of the star-shaped polymers if the number of arms is known and if the polymers have a defined initiator and/or end-group. However, a drawback is that the $^1\text{H NMR}$ measurements do not provide polydispersity indices and can only be applied for rather low molecular weight polymers. On the other hand, a very convenient method to obtain the absolute molecular weight as well as the polydispersity index of any polymer architecture is mass spectrometry and in particular matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS). MALDI has been already shown to be a useful tool for the investigation of large variety of polymers [4], but still has two main limitations: only relative narrow distributed polymers can be investigated [4] and the investigation of high molecular weight polymers is still difficult.

However, the use of preparative size exclusion chromatography can overcome one of these limitations by narrowing the molecular weight distribution of a sample due to

fractionation. Moreover, polymers generally can be analyzed in more detail because each fraction can be characterized by different techniques, as it was for instance shown with ^1H NMR and SEC separately [4-7]. Absolute calibration curves for SEC can be created in combination with MALDI experiments (or other absolute techniques in terms of molar mass determination) in order to obtain the absolute molecular weight of each fraction [5]. The fractionation of polydisperse samples was already used in the literature to obtain fractions with narrower polydispersity indices to subsequently be able to measure MALDI-TOFMS and calculate absolute molecular weight values. Some examples are the fractionation of poly(ethylene terephthalate) and lactones [6], lactides [7], and proanthocyanidins [8]. In these examples, all fractionations were investigated by MALDI-TOFMS in order to obtain absolute SEC calibration curves. Another method to obtain more detailed information of a polymeric architecture is SEC-multi-angle light scattering (SEC-MALS). SEC-MALS is a technique that combines conventional SEC with multi-angle light scattering (MALS). This method can determine the absolute molecular weight as well as the radius of gyration (R_g) and is therefore very valuable for the investigation of branched polymers. Another commonly used method is triple SEC, combining conventional SEC with multi-angle light scattering and a viscosity detector. Here, the viscosity detector measures the intrinsic viscosity as a function of molecular weight and in combination with the light-scattering data detailed information about the polymer architecture can be obtained. A disadvantage of the multi-angle light scattering method is that it is not applicable to rather low molecular weight polymers (low scattering intensity) while SEC in combination with online viscometry is applicable. Therefore, SEC in combination with online viscometry was the method of choice for the investigations reported within this contribution, allowing the determination of absolute molecular weights utilizing the universal calibration method as well as the Mark-Houwink parameters for any polymer [9]. If no suitable standards are available, the absolute calibration curve utilizing SEC with online viscometry allows the accurate determination for all kind of macromolecules, for example biopolymers like pectin, or synthetic ones like poly(vinyl alcohol) and linear block copolymers [10 – 12].

Star-shaped polymeric architectures are especially interesting because of their rheological behavior and high end-group functionality. In particular star-shaped poly(ϵ -caprolactone) (PCL) gained a lot of attraction due to its biodegradability and biocompatibility [13, 14]. PCL degrades in physiological conditions (e.g. in the human body) and therefore applications in drug delivery and the use of PCL as implantable devices are frequently investigated [15]. Moreover, PCL is compatible with a range of other materials so it can be mixed to increase biodegradability or used as plasticizer [16].

In this contribution preparative size exclusion chromatography as well as SEC with online viscometry is used to investigate a series of star-shaped PCL polymers in detail. To obtain information about the polymer architecture, as well as to show application possibilities and discuss limitations of all applied analytical techniques, the fractions are used for further analysis with MALDI-TOFMS to create an absolute calibration curve for SEC analysis for the star-shaped PCL polymers with different M/I ratios. Furthermore, SEC with online viscometry is used to determine the absolute molecular weights of the star-shaped PCL polymers and the Mark-Houwink parameters in DMA using the measured intrinsic viscosities. All obtained results are discussed and compared in detail showing the general ease and usefulness of (offline) hyphenated analytical techniques.

Results and discussion

Recently, we described the synthesis and characterization of a series of star-shaped PCL polymers with different M/I ratios [1]. A star-shaped PCL polymer from this series ($M_n = 10400$ g/mol by ^1H NMR) was subjected to fractionation by preparative size exclusion chromatography resulting in eight narrowly distributed fractions of different molar masses. The fractions were subsequently analyzed by SEC, ^1H NMR, MALDI-TOFMS and SEC with online viscometry. All obtained results were evaluated in detail and compared to one another.

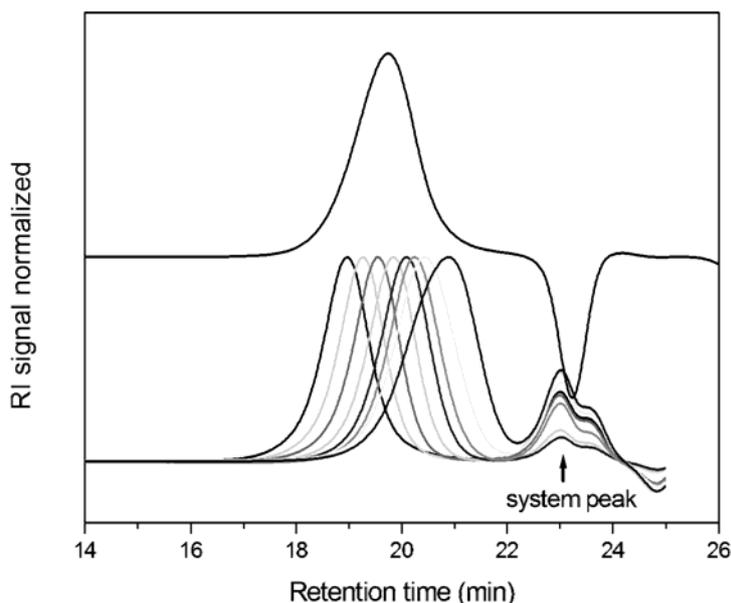


Fig. 1. SEC analysis of the unfractionated PCL star-shaped sample (top) and the obtained fractions by preparative size exclusion chromatography (bottom).

In order to fractionate the PCL star-shaped polymer it was dissolved in THF (50 mg/mL) and 1 mL of this solution was injected onto a PSS Gram preparative 100 Å column. Fractions of equal time width were collected with a connected fraction collector. The star-shaped PCL polymer fractions were then further analyzed with conventional SEC on a Waters HT4 column using a linear PEG calibration. The traces of all fractions revealed narrow molecular weight distributions (PDI ranging from 1.13 to 1.22) covering the complete molecular weight range of the unfractionated sample proving a successful fractionation as shown in Figure 1. As described in the literature, the molecular weights obtained by SEC are lower than expected due to the lower hydrodynamic volume of the star-shaped polymers [1 -3]. Therefore, ^1H NMR was measured for all fractions to calculate the absolute molecular weight for each fraction using the integral of the OH-CH₂-end-group signal at 3.8 ppm in relation to the integrals of the repeat units as it is exemplarily shown for the ^1H -NMR measurement depicted in Figure 2.

The molecular weights of each fraction obtained by ^1H NMR and SEC are plotted in Figure 3 for comparison reasons. Both SEC and ^1H NMR molecular weights of the fractions revealed a linear relation with their retention time on the SEC system indicating that the measurements are performed in the linear range of the column. However, the molecular weights obtained by SEC seems underestimated in

this case due to the smaller hydrodynamic volume of star-shaped polymers if compared to the linear calibration standards as already discussed above.

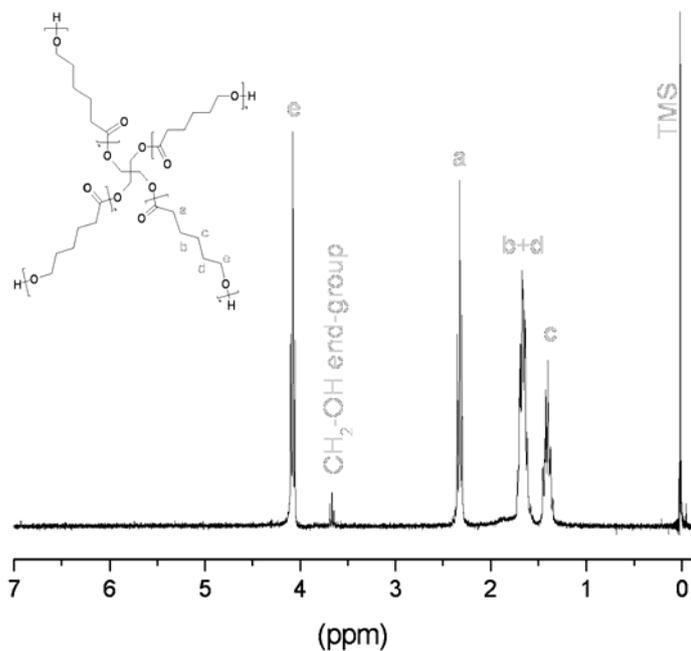


Fig. 2. ¹H-NMR measurement to calculate the absolute molecular weight using the integral of CH₂-end-group at 3.8 ppm in relation to the integrals of the repeat units.

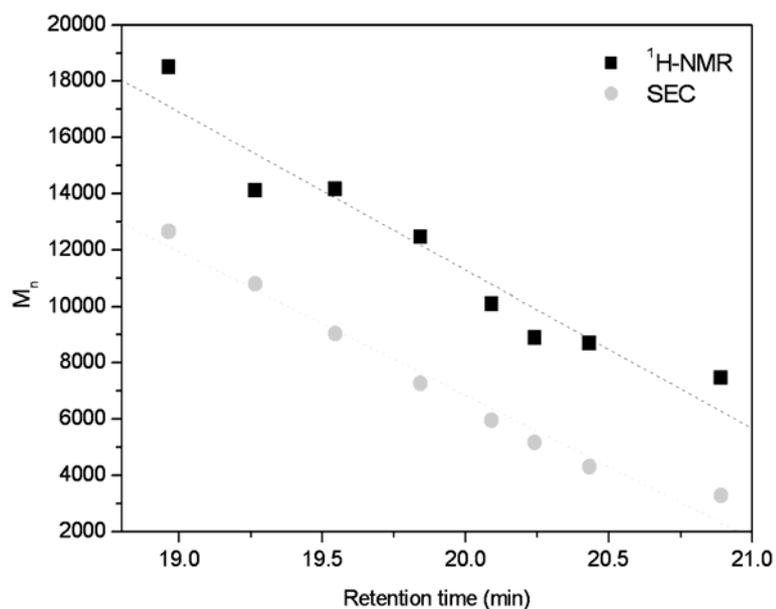


Fig. 3. Molecular weights calculated by ¹H-NMR and SEC for all obtained fractions by preparative size exclusion chromatography.

An additional result of the ^1H NMR investigation revealed that even in the low molecular weight fractions only $\text{OH-CH}_2\text{-CH}_2\text{-R}$ end-groups were observed (and no signal of the pentaerythritol initiator) indicating a quantitative initiation during the polymerization process and therefore similar arm lengths of all four polymer arms. This is the first important result with respect to the usefulness and applicability of the different analytical techniques: ^1H NMR seems to be able to provide correct M_n values and useful structural information, but no polydispersity index information, whereas conventional SEC fails to provide accurate molecular weight data. MALDI-TOFMS is a valuable tool for determining absolute molecular values of a polymer.

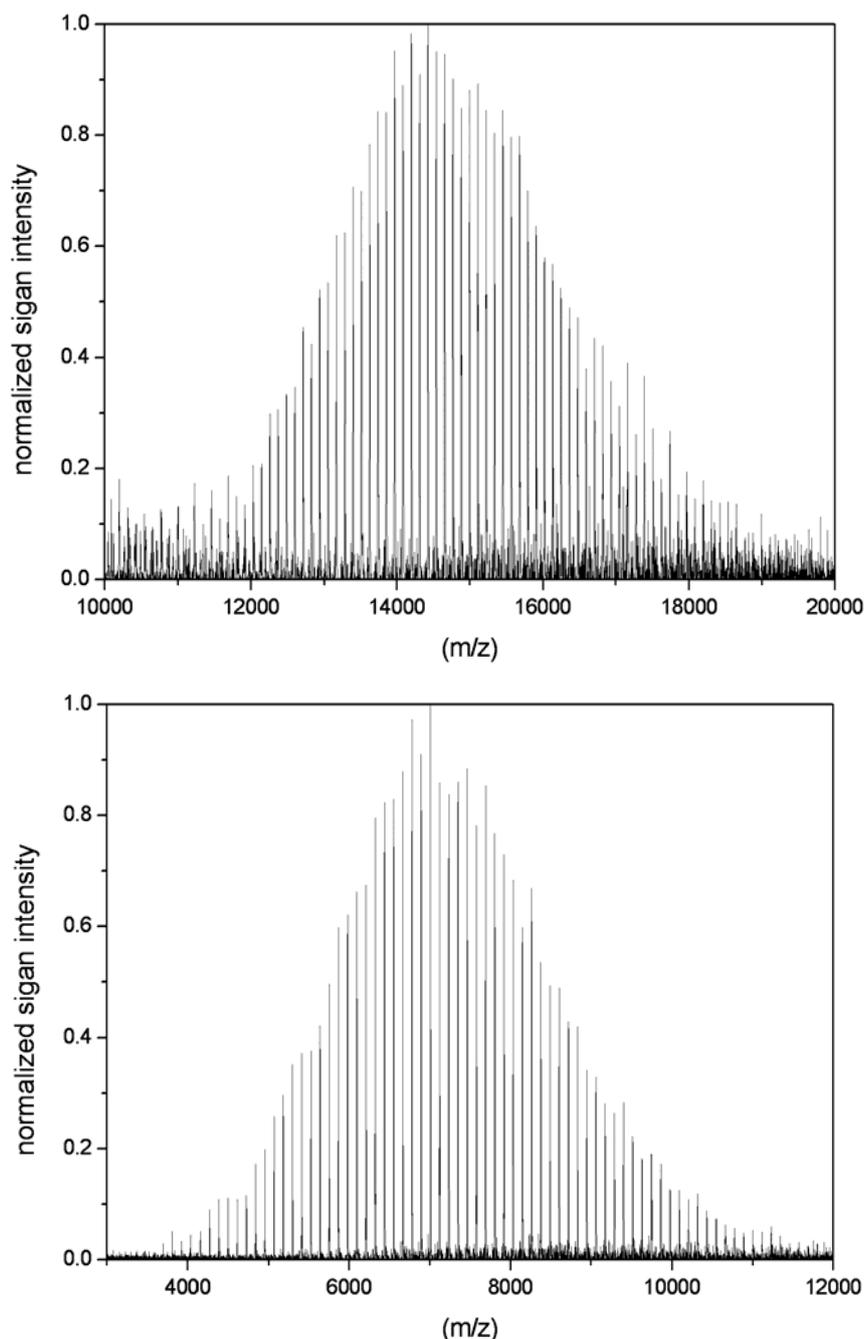


Fig. 4. MALDI experiments of fraction 4 (top) and fraction 8 (bottom) revealing the M_p values.

Therefore, all fractions were investigated in detail by MALDI (examples depicted in Figure 4) revealing the expected 114 Da repeat unit spacing of ϵ -caprolactone; end-group assignments for all fractions were fitted, and the possibility to investigate also higher molecular weight species with good resolution. Moreover, the obtained absolute molecular weight data, in particular the M_p values required for SEC calibrations, in combination with the peak retention times of the fractions on the SEC system were subsequently used to create a new absolute calibration curve for SEC analysis as shown in Figure 5. As the number of arms is the same for all synthesized star-shaped PCL polymers, the hydrodynamic volume is increasing with the number of repetition units per arm and thus the retention time in SEC measurements is decreasing. The star PCL calibration curve in the investigated range revealed to have a linear correlation with a regression coefficient of 0.99. The usefulness of the obtained star PCL calibration curve was tested with the synthesized series of star-shaped PCL polymers with different M/I ratios (number of repetition units).

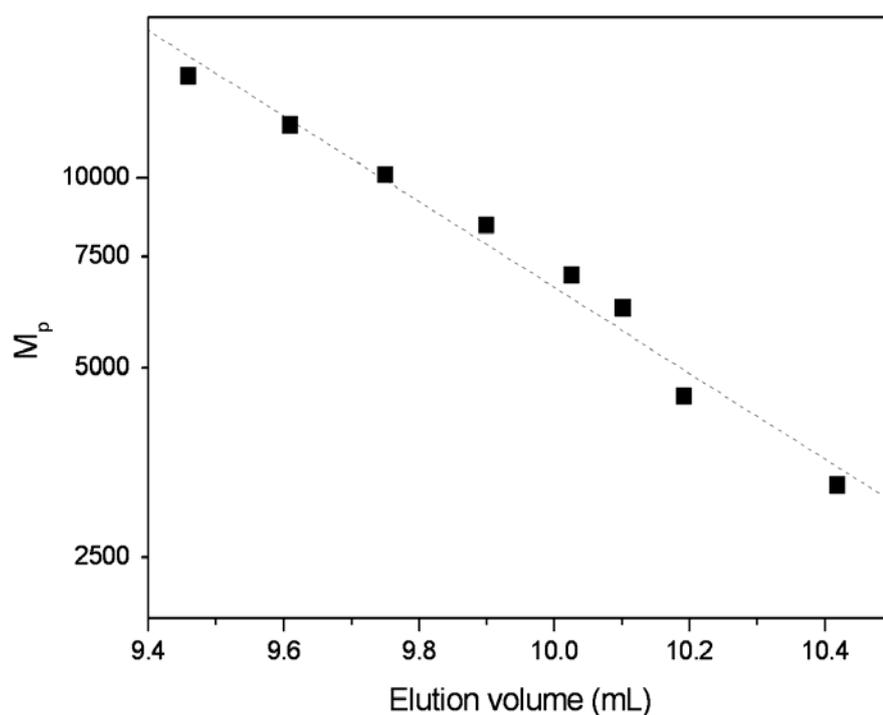


Fig. 5. Absolute calibration curve for star-shaped PCL polymers, M_p 's determined by MALDI are plotted versus the corresponding elution volume from SEC measurements.

Figure 6 compares the molecular weight data obtained from MALDI and the results obtained with the new MALDI (star PCL) calibration. The dotted line represents the theoretical molecular weight. The new measurements with the star PCL SEC calibration are in good agreement with the conventional MALDI measurements revealing that the new calibration is really useful in the determination of the distribution and molecular weight by SEC. The higher molecular weight polymers show a slightly lower molecular weight than expected from theory. This might be explained by a mass discrimination effect during the MALDI experiments of the

higher molecular weight polymers since it is rather difficult to analyze the higher molecular weight polymers by MALDI.

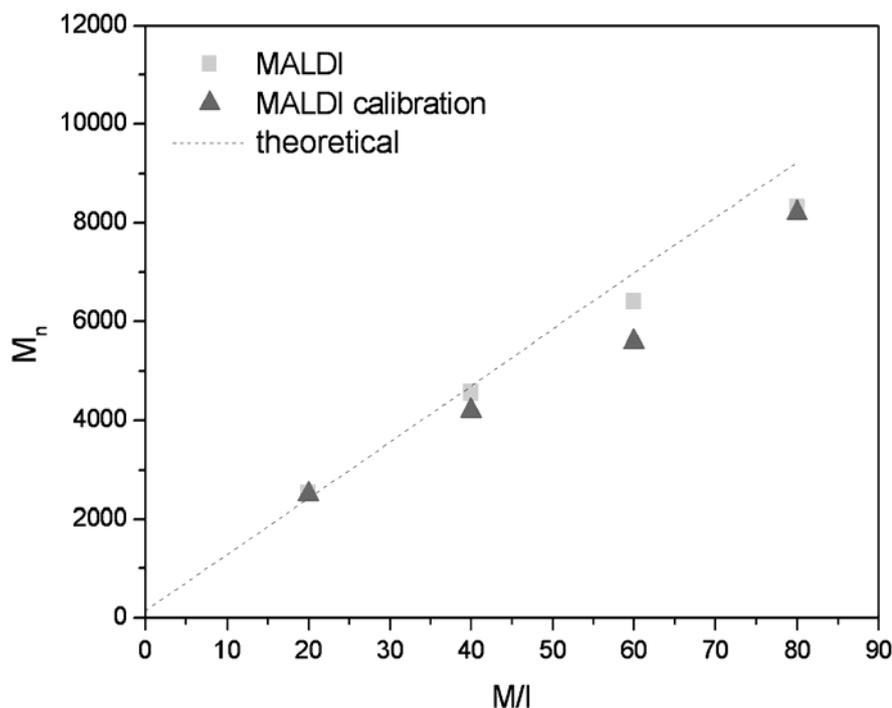


Fig. 6. MALDI results in comparison with the results of the absolute MALDI calibration.

The advantage of SEC calibration, in comparison to the MALDI experiments, is its ease and speed of use and the possibility to use the fractions for the calibration on any SEC system, making good calibration possibilities available to researchers that do not have access to MALDI or other equipments that allow the determination of absolute molecular weights. Moreover, in this way it is possible to use fractionated samples as standards for SEC calibration and any polymer that can be fractionated and analyzed by MALDI can be used as base for new and accurate SEC calibration curves. Another method for the determination of absolute molecular weights of polymers is the use of SEC with online viscometry. SEC with online viscosity measurements utilizing the universal calibration method proved to be a straightforward method for a wide range of different polymeric architectures to calculate the absolute molecular weights and to determine the Mark-Houwink parameters [10 – 12].

Figure 7 depicts the results obtained from the viscosity measurements of the series star-shaped PCL polymers in comparison with the other analytical techniques, such as conventional SEC and ^1H NMR. The results from the online viscosity measurements provide absolute molecular weights utilizing a universal calibration method [17]. Therefore, it is understandable that Figure 7 shows that the obtained values for SEC with online viscosity reveals molecular weights in the same range to those of the ^1H NMR measurements rather than the underestimated values by conventional SEC. Moreover, SEC with online viscosity measurements revealed the Mark-Houwink parameters, using the measured intrinsic viscosities of all fractions. Plotting the $\log M_p$ versus the intrinsic viscosity (see Figure 8) resulted in the Mark-

Houwink plot; the slope of the plot is α and the intercept is equal to $\log K$ according to the Mark-Houwink equation ($[\eta] = K * M^\alpha$). The calculated Mark-Houwink parameters for star-shaped PCL polymers in DMA revealed to be $\alpha = 0.379$ and $K = 0.589$.

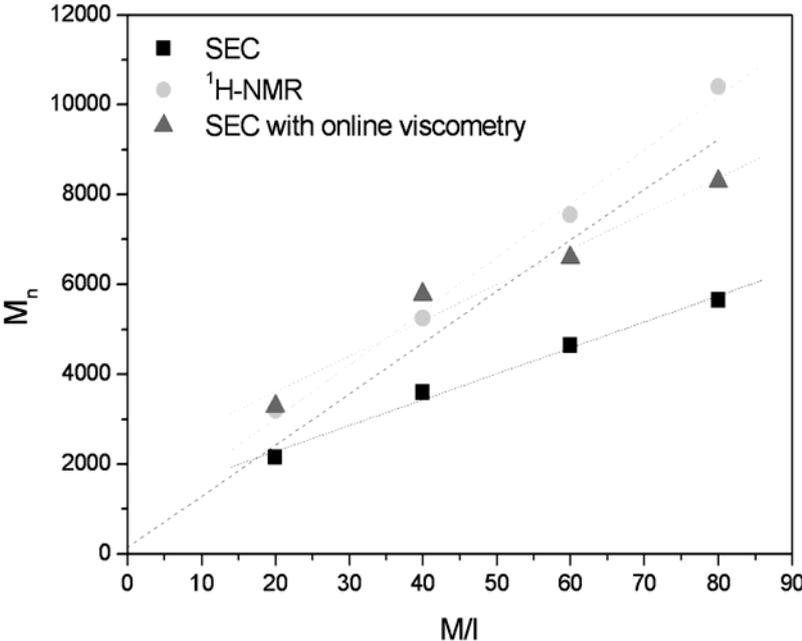


Fig. 7. Results are plotted from the viscosity measurements of the series of star-shaped PCL polymers in comparison with the other analytical techniques, such as SEC and $^1\text{H-NMR}$.

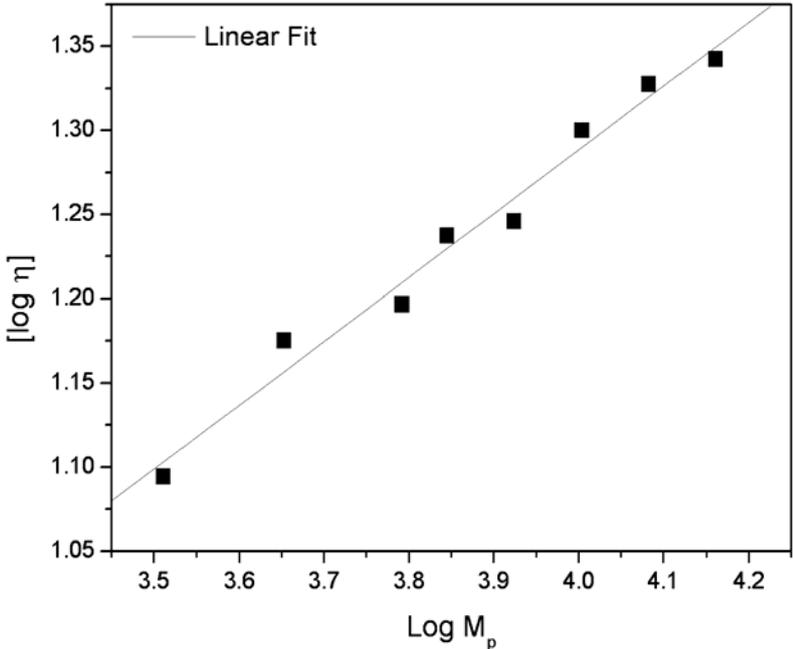


Fig. 8. $\log M_p$ plotted versus the intrinsic viscosity of all analyzed PCL star-shaped fractions. The linear fit reveals the Mark-Houwink parameters for star-shaped PCL polymers in DMA.

Subsequently, the Mark-Houwink parameters can be used for further analysis to calculate the branching factor of the star-shaped polymers. The branching factor (g') is calculated by dividing the intrinsic viscosities of a branched polymer by that of a linear one with the same molecular weight. If the branching factor equals 1, the polymer is linear and if the branching factor becomes smaller, there are more branches (arms) present. The branching ratio can be further used for the calculation of average number of branch units per molecule or number of arms in star-branched polymers [18]. The calculated branching factor for the star-shaped PCL polymers is varying from 0.63 to 0.70, indicating branched polymer architecture. For more accurate branching factors, on-line multi angle light scattering should be measured (so called MALLS) in combination with SEC-viscometry. Nevertheless, the online-viscosity hyphenation can provide first insights into the polymeric architectures in combination with accurate molecular weight values in a straightforward fashion.

In summary, the discussed results clearly show that characterization via size exclusion chromatography and successive off-line hyphenated techniques open ways to study the molecular weight as well as architecture of a polymer in detail. In the future this approach will be extended to copolymers and the integration of even more off-line characterization, such as FT-IR, in order to obtain composition distributions of polymers in an easy and straightforward manner.

Experimental part

The synthesis of the series star-shaped PCL polymers is already described in literature [1].

Instrumentation

All MALDI experiments were carried out on a Voyager-DETM PRO BiospectrometryTM Workstation time-of-flight mass spectrometer using reflector mode for operation. All spectra were obtained in the positive ion mode. Ionisation was performed with a 337 nm pulsed nitrogen laser. Samples were prepared with a multiple-layer spotting technique utilizing dithranol as matrix as described previously [19].

NMR spectra were measured on a Bruker Mercury 400 NMR spectrometer in CDCl₃. The chemical shifts were calibrated to tetramethylsiloxane.

Size exclusion chromatography is measured on a Waters GPC system consisting of an isocratic pump, solvent degasser, column oven, 2414 refractive index detector, 717plus autosampler and Styragel HT 4 GPC column with precolumn installed. The eluent was dimethylformamide with 5 mM NH₄PF₆ as additive at a flow speed of 0.5 mL/min and a column temperature of 50 °C. Narrowly distributed linear poly(ethylene glycol) standards were used for calibration.

Preparative size exclusion chromatography was performed on an Agilent system consisting of a Agilent 1100 series Control Module, a Agilent 1100 series Isocratic Pump, A Agilent 1100 series RID refractive index detector, a Agilent 1100 series Manual Injector and a PSS GRAM 10 μ , 20 x 300 mm preparative 100 Å column utilizing THF as eluent at a flow rate of 3 mL/min.

Viscosity measurements were performed on a Shimatzu GPC, equipped with a system controller SCL-10Avp, a LC-10AD pump, a RID-10A refractive index detector, a UV/VIS detector DPD-10A, a PSS ETA-2010 differential viscometer, degasser DGU-14A and a CTO-10A column oven and two PSS GRAM 10 μ , 8 x 300 mm, 1000

/ 30 Å columns utilizing DMA/LiCl as eluent. The universal calibration is prepared by using readycal PS standards.

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