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ORIGINAL PAPER

Sedimentation studies of single and bi-component polystyrene solutions in analytical ultracentrifugation by comparison of two analysis methods: effect of polymer concentration

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Abstract Sedimentation analysis with interference detection optics was carried out for pure polymer as well as 1:1 polymer mixture samples as a function of polymer concentration in analytical ultracentrifuge. The data analysis was carried out using analysis programs of Sedfit and VelXLAI to elucidate the advantages and limitations of the analysis methods. The concentration dependency of the sedimentation coefficient was observed and the distributions became narrower on increasing the polymer concentration in both the methods. The peak sedimentation coefficients at different concentrations for both pure polymer as well as polymer mixture samples were same in both the methods though the sedimentation coefficients of the components in the mixture were slightly different from the sedimentation coefficient of the same component, when measured alone. The programs were observed to generate similar information in the case of pure polymer sample; however, in more complex polymer mixture sample, the Sedfit program was observed to have significant difference in the shape of the sedimentation coefficient distributions as well as in the amount of the components detected.

Keywords VelXLAI · Sedfit · Sedimentation · Ultracentrifugation · Mixture

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Introduction

Analytical ultracentrifugation is a method of choice for colloid characterization owing to its high statistical capability and versatility to analyze a wide spectrum of polymer systems [1–3]. A sedimentation velocity experiment using interference detection optics is the commonly used method where the change in the sample concentration as a function of radius of the ultracentrifugation cell and time of sedimentation is recorded [4–8]. This sedimentation velocity data are used to define the distribution of the sedimentation coefficient, s which can then be converted to required information like molecular weight distribution or particle size distribution.

A number of data analysis programs have been developed in the recent years to transform the sedimentation velocity data into sedimentation coefficient distributions and subsequently into particle size or molecular weight information. One of the widely used analysis method is Sedfit and is based on generating numerical solutions to the Lamm equation [9–13]. This method is based on a boundary model approach for sedimentation which then generates information on molecular weight or particle size distribution. It is also possible in this method to analyze polydisperse as well as multi-component systems [14–16]. The analysis requires following assumptions:

(a) sedimentation coefficient,
(b) frictional coefficient, and
(c) the partial specific volume for the solute.

This information is required to generate finite element solutions for a large number of species with varying s values. The analysis is followed by the adjustment of the generated solutions to experimental data by maximum entropy regularization, which generates a sedimentation coefficient distribution $g(s)$. Thus, the method requires beforehand knowledge of partial specific volume and the frictional ratio of the sample, which can be a limitation in some cases.

Gosting Fujita Lechner method (incorporated into analysis program of VelXLAI) for colloid analysis is free from any model assumptions The sedimentation coefficient distribution $g(S, C, t)$ is determined according to [17–20]:

$$S(C,t) = (\mathrm{d}r/\mathrm{d}t)/(\omega^2 r) = (1/\omega^2)\ \mathrm{d}\ \ln r/\mathrm{d}t = \ln(r/r_\mathrm{m})/\left(\int \omega^2 \mathrm{d}t\right)$$

$$\mathrm{g}(S,C,t) = (1/C_0)\,[\mathrm{d}C(r,t)/\mathrm{d}r]\,(r/r_\mathrm{m})^2 r \int \omega^2 \mathrm{d}t$$

where C_0 is the initial concentration of the particles and $C(r, t)$ the concentration at distance r and running time t, $r =$ distance from the axis of rotation; $r_\mathrm{m} =$ distance of the meniscus from the axis of rotation; $= 2\pi N =$ angular velocity. The details on the method have also been provided elsewhere [13, 21].

The aim of this study was to compare the strengths and limitations of above mentioned data analysis programs in the interference optics detection mode. Sedimentation studies on single component as well as bi-component polystyrene solutions in methylethylketone (MEK) were performed. The effect of polymer concentration on the generated sedimentation coefficient and sedimentation coefficient distributions was studied in both the data analysis programs. Relative fractions of the components of the polymer mixture detected in these programs as a function of polymer concentration were also compared with each other.

Materials and methods

Two standard polystyrene samples with molecular weights in the range of ~80,000 and ~350,000 g/mol were commercially procured and were respectively designated as PS-1 and PS-2. MEK was used as organic solvent to generate polymer solutions with different polymer concentrations. Sedimentation velocity experiments in the interference detection optics were performed in an OPTIMA XL-I analytical ultracentrifuge from Beckman Coulter (temperature 25 °C, wavelength 675 nm). A rotational speed of 40,000 rotations per minute and a scan time interval of 10 s were used for the analysis of polymer samples as well as their mixtures. A dn/dc value of 0.21 cm^3/g was measured for the polymer samples in the Bellingham refractometer. Polymer concentrations ranging from ~1 to ~5 g/L were used to study the concentration dependency of the sedimentation coefficient. The sedimentation velocity data obtained from the analytical ultracentrifuge were analyzed using analysis programs of Sedfit and Gosting Fujita Lechner (GFL) or VelXLAI method. The internal parameters of both of analysis program were fine-tuned so as to achieve a good fit of the experimental data.

Results and discussion

Molecular characteristics of the polymer chains like molecular weight distribution (generated from sedimentation coefficient distribution) directly influence their commercial applications thus requiring accurate characterization. It is also important to know the relative amounts of the components of different molecular weights in the polymer mixture samples to relate this information to the performance. To accomplish this, two different data analysis programs of Sedfit and Gosting Fujita Lechner method (or VelXLAI) in analytical ultracentrifugation were chosen with the aim to compare the capability and accuracy of these methods for the sedimentation analysis of the polymer solutions. Single polymer as well as mixture of two polymers were analyzed as a function of polymer concentration in the solution. Sedimentation coefficient distributions as well as percentages of components detected in the mixture by both the methods were compared.

Figure 1 shows the raw sedimentation profiles of the pure polymer PS-2 and its 1:1 weight mixture with PS-1 polymer as a function of the cell radius and sedimentation time at a polymer concentration of 2.9 and 3.0 g/L, respectively. The presence of two sedimentation plateaus in the mixture sample confirms the presence of two families of polymer chains. The scans in the sedimentation plateau corresponding to low molecular weight polymer PS-1 (lower plateau in Fig. 1b) were observed to be more flat or sagging in nature with lower slope as compared to

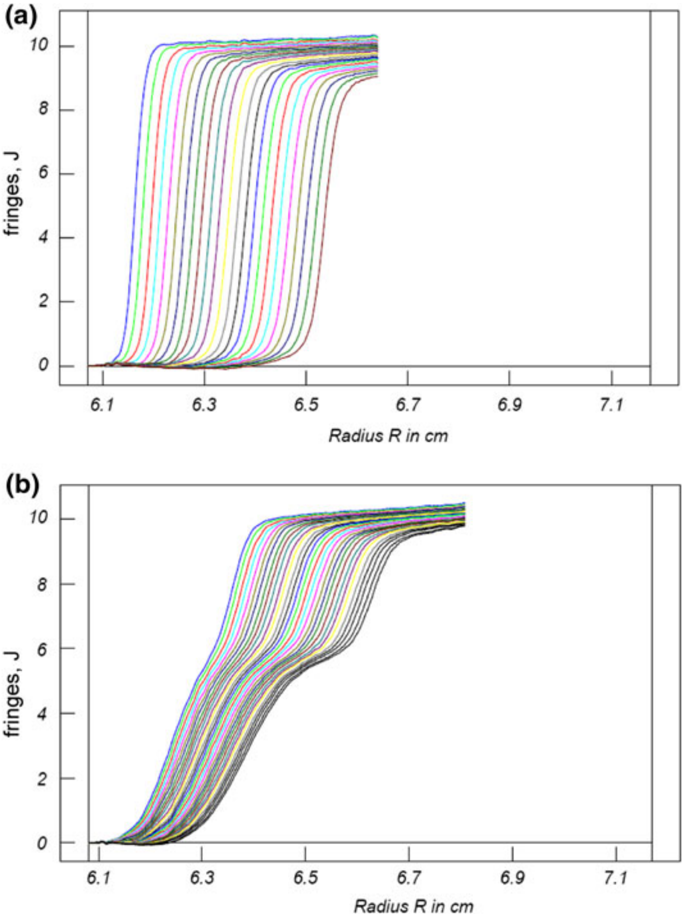


Fig. 1 Raw sedimentation profiles of **a** polystyrene sample PS-2 (2.9 g/L) and **b** 1:1 mixture of polystyrene samples PS-1 and PS-2 (3.0 g/L), when measured with interference optics

the scans of polymer PS-2 (steep slope of the scans in this case). It indicated that the chains in PS-1 may have a more polydisperse nature than the chains in PS-2 polymer.

Figure 2 shows the differential and cumulative sedimentation coefficient distributions for the polymer sample PS-2 as a function of polymer concentration when analyzed with VelXLAI program. The single distribution of the sedimentation coefficient confirmed the presence of one family of polymer chains in the sample. It can be seen that the distributions shifted to lower sedimentation coefficient values as a function of polymer concentration, indicating the concentration dependency of the sedimentation coefficient in the MEK solvent [22]. Polymer concentrations of 1.0, 2.0, 2.9, 4.1, and 5.1 g/L were used, and the peak sedimentation coefficient for these polymer concentrations was observed to be 14.47, 13.49, 12.61, 11.58, and 10.79 Sved, respectively. Apart from that, the distributions were also observed to become

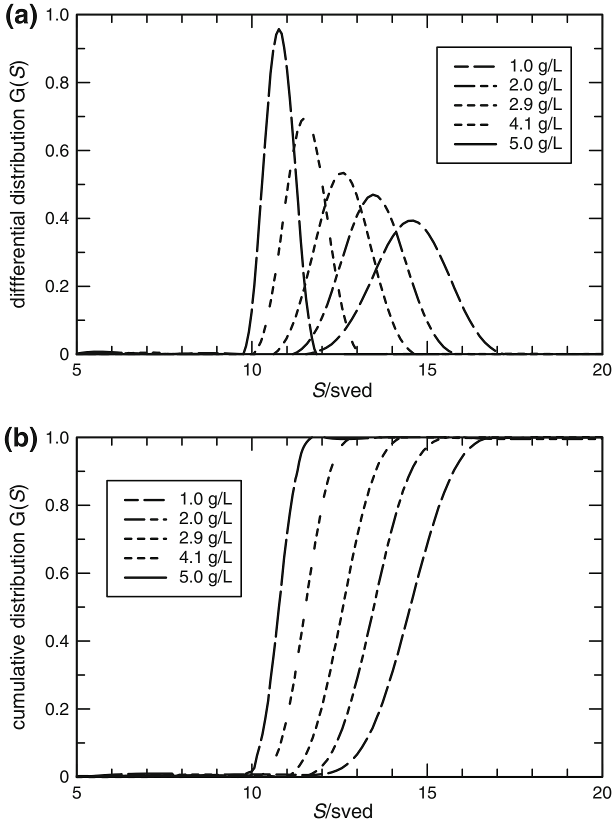


Fig. 2 **a** Differential and **b** cumulative sedimentation coefficient distributions of polystyrene sample PS-2 at different polymer concentrations using interference optics for measurement and VelXLAI program for data analysis

narrower on increasing polymer concentration. This effect was also visible from the cumulative distributions of the sedimentation coefficient as the slope of the curve became steeper with increasing concentration. Figure 3 shows the differential and cumulative sedimentation coefficient distributions of the polymer at the same concentrations as shown in Fig. 2, but in this case the data analysis was carried out with Sedfit program. The concentration dependency of the sedimentation coefficient was also confirmed in this case, and the distributions also became narrow as a function of increasing polymer concentration similar to the observation with the VelXLAI analysis. The peak sedimentation coefficient values as a function of increasing polymer concentration in Sedfit analysis were observed to be 14.6, 13.57, 12.6, 11.60, and 10.75 Sved, respectively, which were in close proximity to the values observed with VelXLAI program. The ranges of the minimum and maximum sedimentation coefficient values obtained by the two programs were also similar, but Sedfit program showed slight tailing at both lower and higher end of the sedimentation coefficient distributions of the polymer samples.

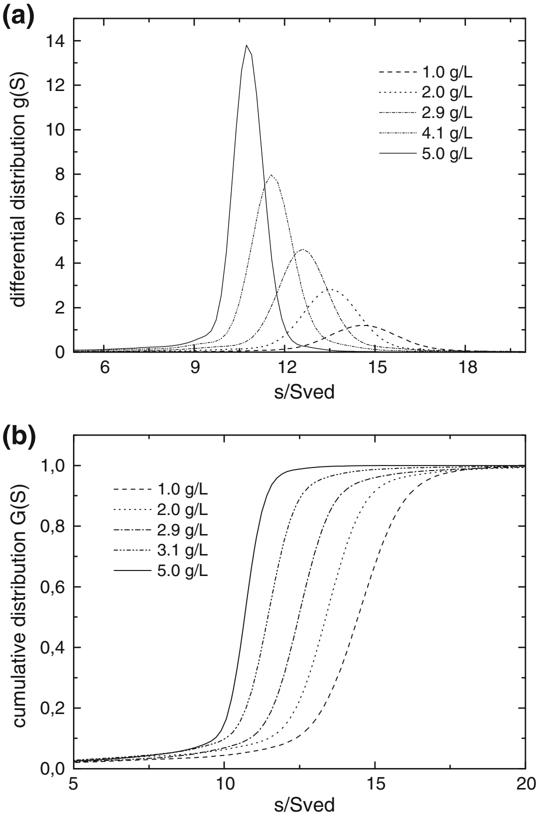


Fig. 3 **a** Differential and **b** cumulative sedimentation coefficient distributions of polystyrene sample PS-2 at different polymer concentrations using interference optics for measurement and Sedfit program for data analysis

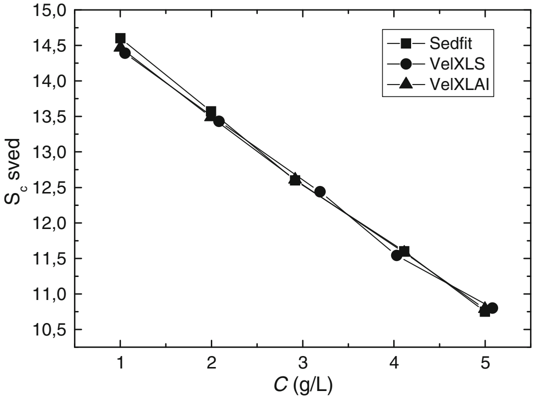


Fig. 4 Plot of sedimentation coefficient S_c as a function of polymer concentration for polystyrene sample PS-2 polymer using Sedfit, VelXLAI, and VelXLS data analysis programs

Figure 4 also demonstrates the correlation of peak sedimentation coefficient as a function of polymer concentration for both VelXLAI and Sedfit programs. In addition to the data from VelXLAI and Sedfit programs, the results from another analysis program VelXLS based on Schlieren detection optics have also been added

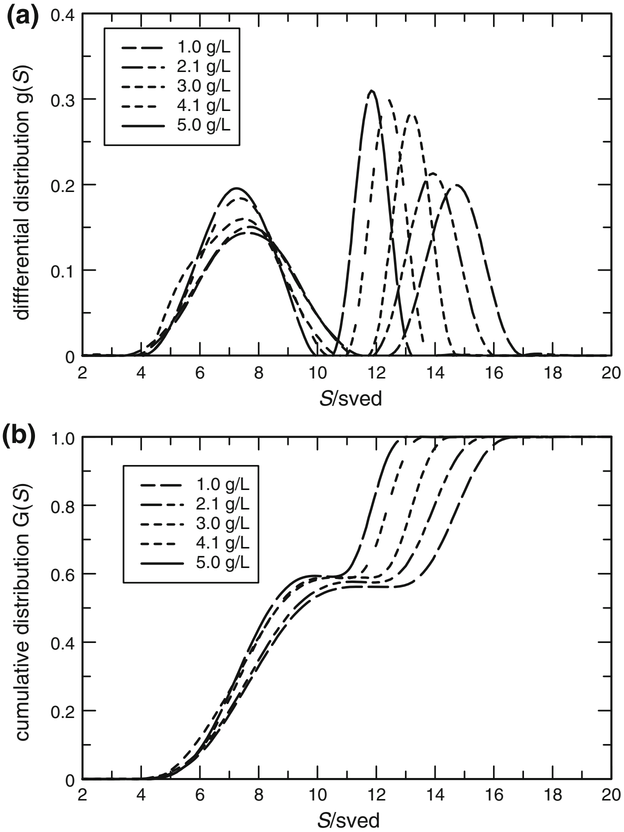
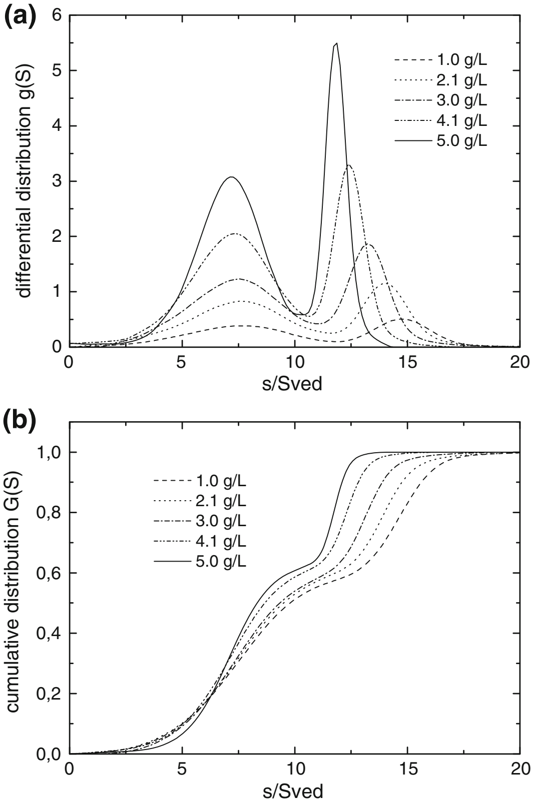


Fig. 5 **a** Differential and **b** cumulative distributions of sedimentation coefficient for the 1:1 mixture of polystyrene samples PS-1 and PS-2 at different concentrations of 1.0, 2.0, 3,0, 4.1, and 5.0 g/L. Interference optics were used for data acquisition, and VelXLAI program was used for data analysis

for comparison [22]. The sedimentation coefficients at different polymer concentrations were fairly close to each other for all the programs confirming almost similar results from these programs for the sedimentation analysis of single component polymer solutions.

Though the sedimentation analysis of the single component polymer solutions by Sedfit and VelXLAI yielded similar results, it was of interest to apply the procedure on a slightly complex system, e.g., polymer mixtures. Figure 5 shows differential and cumulative distributions of sedimentation coefficient of a 1: 1 PS-1 and PS-2 polymer mixture as a function of polymer concentration when analyzed with VelXLAI program. For simplicity, only 1:1 mixture of PS-1 and PS-2 was considered, but the mixtures with lower amounts of lower or higher molecular weight components should also be analyzed to confirm the benefits of each method. Similar to the case with single component polymer sample, polymer concentrations of 1.0, 2.1, 3.0, 4.1, and 5.0 g/L were used. The presence of two distributions in the curves confirmed the presence of two families of polymer chains. The concentration

Fig. 6 **a** Differential and **b** cumulative distributions of sedimentation coefficient for the 1:1 mixture of polystyrene samples PS-1 and PS-2 at different concentrations of 1.0, 2.0, 3,0, 4.1, and 5.0 g/L. Interference optics were used for data acquisition, and Sedfit program was used for data analysis



dependency of the sedimentation coefficient was also present with similar shifting of sedimentation coefficient to lower values as observed earlier for PS-2 samples. The peak sedimentation coefficient values of the PS-2 fraction in the polymer mixture were however slightly different from the single component PS-2 sample indicating slight change in the sedimentation profile of the polymer in the presence of other polymer. Figure 6 similarly describes the differential and cumulative sedimentation coefficient distributions of the same 1:1 PS-1 and PS-2 polymer mixture as a function of polymer concentration when analyzed with Sedfit program. Bimodal distributions, similar to VelXLAI, were observed and also showed similar concentration dependency of the sedimentation coefficient. In this case too, the peak sedimentation coefficient values of the PS-2 fraction were slightly different from the values observed when only PS-2 was measured confirming the observation from the VelXLAI analysis. Similar to the pure PS-2 sample, tailing at the beginning as well as end of the sedimentation coefficient distributions was also observed with Sedfit program, whereas, no such tailing of the sedimentation coefficient distribution was observed in VelXLAI program. Apart from that, the curves at the intersections of the distributions corresponding to the PS-1 and PS-2 did not touch the *x*-axis in Sedfit analysis, whereas, a complete separation was observed in VelXLAI analysis

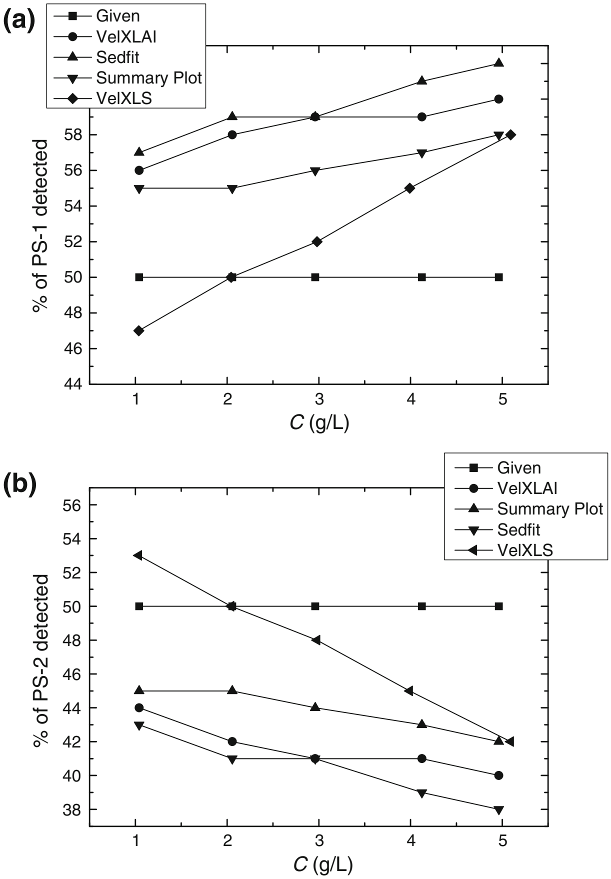


Fig. 7 Percentage of polystyrene samples **a** PS-1 and **b** PS-2 detected in the various analysis programs as a function of polymer concentration

between the two distributions indicating probably better differentiation between the two fractions.

Apart from sedimentation coefficient, it is also important in a mixture sample to analyze the relative amount of mixture fractions detected by the data analysis program. This information is directly relatable to the performance for the material, thus, an accurate characterization of the fractions in the sample is necessary. Of course, owing to the Johnston–Ogston effect in the multi-component systems where the sedimentation coefficient has concentration dependency, the slow sedimenting component accumulates at the first sedimenting boundary and the corresponding contribution of the slower component is larger than the signal created by the faster component [3]. Thus, the relative concentrations of the both components may be incorrect as contribution of the slower component may be high and no analysis program can be expected to detect exact 1:1 concentration of the components for a 1:1 polymer mixture. However, it is still possible to compare the relative performance of two analysis programs in detecting the amount of mixture fractions and as Johnston–Ogston effect is insignificant at smaller polymer concentrations, the relative comparison of the analysis programs at the lower polymer concentration can be more accurate. Figure 7 shows the fractions of PS-1 and PS-2 detected by the analysis programs as a function of polymer concentration. For comparison, analysis with VelXLS program was also included [22]. The values from the summary plot (i.e., from the raw sedimentation data) were also plotted. All the detected values were compared with the initially used 1:1 concentration of components. The presence of Johnston-Ogston effect in all the analysis methods was confirmed as the amount of detected fraction of low molecular weight PS-1 was always (except one concentration in VelXLS) higher than 50% and similarly, amount of high molecular weight PS-2 fraction was lower than 50%. However, different analysis programs yielded different values. Maximum deviation in the detected values and given values was observed in Sedfit with detected fractions of 57, 59, 59, 61, and 62% for PS-1 fraction as a function of increasing concentration. VelXLAI program was better than the Sedfit analysis as values of PS-1 and PS-2 fractions more near to ideal 50% as compared to Sedfit were observed. Thus, for the mixture sample, the programs VelXLAI and Sedfit had more differences as compared to single component polymer samples, and the VelXLAI analysis was better than the Sedfit analysis in detecting the relative amount of components in the polymer mixture.

Conclusions

Two data analysis programs of VelXLAI and Sedfit were compared with each other for the sedimentation analysis of pure polymer and polymer mixture samples as a function of polymer concentration. In the case of pure polymer sample, the analysis methods provided similar peak sedimentation coefficient values and sedimentation coefficient distributions. The Sedfit distributions differed slightly as tailing at the beginning and end of the distributions was observed. In the bi-component sample, the distributions with Sedfit also had the tailing as observed for the pure polymer and the intersection between the two distributions did not touch complete zero, whereas a clear separation between the two fractions was observed in the case of VelXLAI analysis. The amount of detected fractions in the bi-component sample also differed in the two programs and VelXLAI was observed to detect the amount of fractions more near to the given values than the Sedfit analysis, though Johnston–Ogston effect led to the observation of higher contribution of low molecular components in all the cases.

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