The Polydispersity Ratio (PDR) and its Application for the Characterization of Poloxamers

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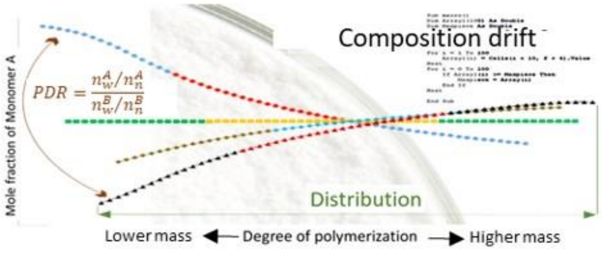


Abstract

The data processing and visualization methods are of paramount importance in the mass spectrometry of copolymers. To determine the copolymer composition, in this article, a robust algorithm is proposed for the compositional assignment and for the estimation of the relative abundance of each species present in copolymer mass spectra. Our home-made software enables the accurate calculation of the compositional drift, i.e., the variation of copolymer composition with the polymer chain lengths. Furthermore, we introduce a novel copolymer quantity, namely the polydispersity (also called as dispersity) ratio (PDR) of the comonomers, and establish a characteristic relationship between the shape of the composition drift curves and the PDR values. This relation allows, for instance, a quick visual recognition of the presence of diblocks in a triblock copolymer by means of mass spectrometry. Our approach is demonstrated by the analysis of various poloxamers, i.e., polyethylene oxide (PEO) - polypropylene oxide (PPO) block copolymers up to the average molecular weight of approximately 4000 g/mol. The determined number-average molecular weights and the ethylene oxide contents were also confirmed by nuclear magnetic resonance (NMR) spectroscopy. Dynamic light scattering (DLS) experiments revealed that small variations in the copolymer composition significantly affect the properties of the copolymer.

Keywords: Mass spectrometry, poloxamer, copolymers, composition drift, polydispersity ratio (PDR), dynamic light scattering (DLS), critical micelle concentration (CMC)

Introduction

The introduction of soft ionization methods such as the matrix-assisted laser desorption/ionization (MALDI) [1,2] and the electrospray ionization (ESI) [3], has opened the way for the mass spectrometric characterization of intact polymers and copolymers [4-8]. Unlike the traditional techniques such as gel permeation chromatography (GPC), nuclear magnetic resonance (NMR) and infrared (IR) spectroscopies those of which all measure only the average, bulk properties of polymers, mass spectrometry (MS) is capable of providing information on the individual polymer chains. However, the interpretation of the copolymer mass spectra, in general, is not a straightforward task due to the following issues: (i) the presence of huge number of *m/z* peaks and (ii) isobaric compositions, (iii) occurrence of overlapped peak series and (iv) isotope peaks, (v) the relatively low resolution of commercial analyzers at high *m/z* region. Therefore, not only the high resolution and mass accuracy, but the data processing and visualization methods play also important roles in the characterization of copolymers [9-11]. Thus, several methods and algorithms have been introduced to assign elemental composition to the individual peaks present in copolymer mass spectra [12-14]. Once the abundance of each copolymer chain is determined, the accurate molecular weight averages M_n (number-average), M_w (weight-average), and the polydispersity (IUPAC suggest the use of dispersity instead, however polydispersity is also a commonly used term) index (M_w/M_n) can be calculated. However, to understand the correlation between the copolymer structure and the copolymer`s properties, it is not enough just to determine the molecular weight and molecular weight distributions, but the knowledge of the variation of composition at different polymer chain lengths (called the "compositional drift"), and the weight of copolymer chains at each composition (the compositional distribution) [15] is also of great importance. Thus, the copolymer properties can be tailored by controlling the composition drift characteristics, therefore, mapping of composition drift can greatly aid polymer manufacturers, processors and end-users [16-18].

In this paper we propose a robust algorithm for the compositional assignment and for the estimation of the relative abundance of all molecular species present in copolymer mass spectra that allows a comprehensive characterization of the copolymer composition, for example, the calculation of the composition drift. We demonstrate our new approach by the analysis of various poloxamers, i.e., ethylene oxide (EO) - propylene oxide (PO) block copolymers. The amphiphilic block copolymers consisting of polyethylene oxide (PEO) and polypropylene oxide (PPO) blocks arranged in a triblock (PEO-PPO-PEO) structure are widely used as nonionic surfactants in industrial and domestic applications, cosmetics, pharmaceuticals and so on [19-21]. Due to the presence of both hydrophilic (PEO) and hydrophobic predominant (PPO) moieties, these block copolymers can form aggregates in aqueous solutions [22]. We report herein that small variations in the copolymer composition determined by our method, are reflected in different aggregation behavior of these block copolymers as determined by dynamic light scattering (DLS) measurements. In addition, to the best of our knowledge, we propose a new copolymer quantity for the first time, namely the polydispersity ratio (PDR) of the comonomers and establish a characteristic relationship between the shape of the composition drift curves and the PDR values. We will also demonstrate that the composition drift curve visually indicates the presence of diblocks in a triblock system.

Experimental

Chemicals

The PE 6400 (sample *P1*), RPE1740-1 (sample *P3*), RPE1740-2 (sample *P4*), RPE3110 (sample *P6*), PE8100-1 (sample *P8*) and PE8100-2 (sample *P9*) copolymers were generous gift from BASF (Ludwigshafen, Germany). RPE1740-1 and PE8100-1 samples were received in 2006, while RPE1740-2 and PE8100-2 samples were obtained in 2021. The poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (sample *P2*) and poly(propylene glycol)-block-poly(ethylene glycol)-block-poly(propylene glycol) (sample *P5* and sample *P7*) were purchased from Merck (Darmstadt, Germany). Methanol was received from VWR International (Leuven, Belgium). Water was purified by a Direct-Q water system (Millipore, Molsheim, France). Table 1 lists the investigated samples with the manufacturer's specifications.

Table 1. The list and type of the investigated samples.

Sample name	Type	Origin	EO content m/m% (based on provider)	Block sequence
P1	PE6400	BASF	40	EO-PO-EO
P2	PE6400 analogue	Merck	40	EO-PO-EO
P3	RPE 1740-1	BASF	40	PO-EO-PO
P4	RPE 1740-2	BASF	40	PO-EO-PO
P5	RPE1740 analogue	Merck	40	PO-EO-PO
P6	RPE3110	BASF	10	PO-EO-PO
P7	RPE3110 analogue	Merck	10	PO-EO-PO
P8	PE8100-1	BASF	10	EO-PO-EO
P9	PE8100-2	BASF	10	EO-PO-EO

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS)

An Autoflex Speed MALDI-TOF MS instrument (Bruker Daltonik, Bremen, Germany) was applied for all the measurements. Reflectron mode was used where the ion source voltage 1, ion source voltage 2 reflector voltage 1 and reflector voltage 2 were 19 kV, 16.65 kV, 21 kV and 9.55 kV, respectively. The instrument is equipped with a solid phase laser (355 nm). All the spectra were internally calibrated, applying different polyethylene glycol homopolymers or their mixtures. The samples were prepared with 2,5-dihydroxybenzoic acid (DHB) matrix and sodium trifluoroacetate (NaTFA) ionizing agent. Methanol was used as a solvent, the concentrations of the matrix, samples and ionizing agents were 20 mg/mL, 10 mg/mL and 5 mg/mL, respectively. The matrix, sample and the ionizing agent solutions were mixed in the ratio of 5:2:1, respectively.

Dynamic Light Scattering (DLS)

The hydrodynamic size and size distributions of aggregates in aqueous solution were determined by dynamic light scattering (DLS). The samples were filtered through a 0.45 μm syringe filter to eliminate dust or other impurities. DLS measurements were performed using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) equipped with a He-Ne laser (633 nm) at 37°C and at a detector position of 175°. The Z-average size and polydispersity index (PDI_{DLS}) were calculated by cumulants analysis. The distribution by intensity of particle sizes were determined by multiple exponential fit.

All the copolymers were dissolved in water in a concentration of approximately 4 mM. These stock solutions were used for the preparation of DLS samples, concentrations are shown in the Supporting information (Table S1. and Table S2.). The diluted solutions were filtered and stored at 5 °C at least for 24 h. The samples were thermostated at 37°C before the analysis at least for 24 h.

Nuclear magnetic resonance measurements

NMR measurements were acquired on a Bruker Avance II 500 MHz spectrometer equipped with a 5 mm z-gradient TXI probe. Quantitative 1D ^{1}H NMR experiments were performed at 300 K with a 45 degree ^{1}H excitation pulse, a relaxation delay of 15 s, spectral width of 14.97 ppm, 32k total data points and 16 scans. Bruker TopSpin 3.0 software was used for NMR spectral processing and integration. For the NMR measurements 30 mg of the sample was dissolved in 600 μL $CDCl_3$ to which 10-fold molar excess of trifluoroacetic anhydride was added. The solutions were kept at room temperature for 20 h prior to analysis. The NMR spectra of the samples and the peak assignments are shown in Figures S1−S9 in the Supporting Information.

Results and Discussion

Figure 1 shows the MALDI-TOF mass spectra of two amphiphilic triblock copolymers consisting of polyethylene oxide and polypropylene oxide (PEO–PPO–PEO) with approximately 40 wt% EO content: ***a)*** Pluronic PE6400 from BASF (sample *P1*) and ***b)*** the PEO–PPO–PEO triblock copolymer from Merck (sample *P2*) sold as an analogue of PE6400. Additional MALDI-TOF MS spectra of triblock copolymers with various EO contents and number-average molecular weights (M_n) are given in Figures S1-S7 in the Supporting Information.

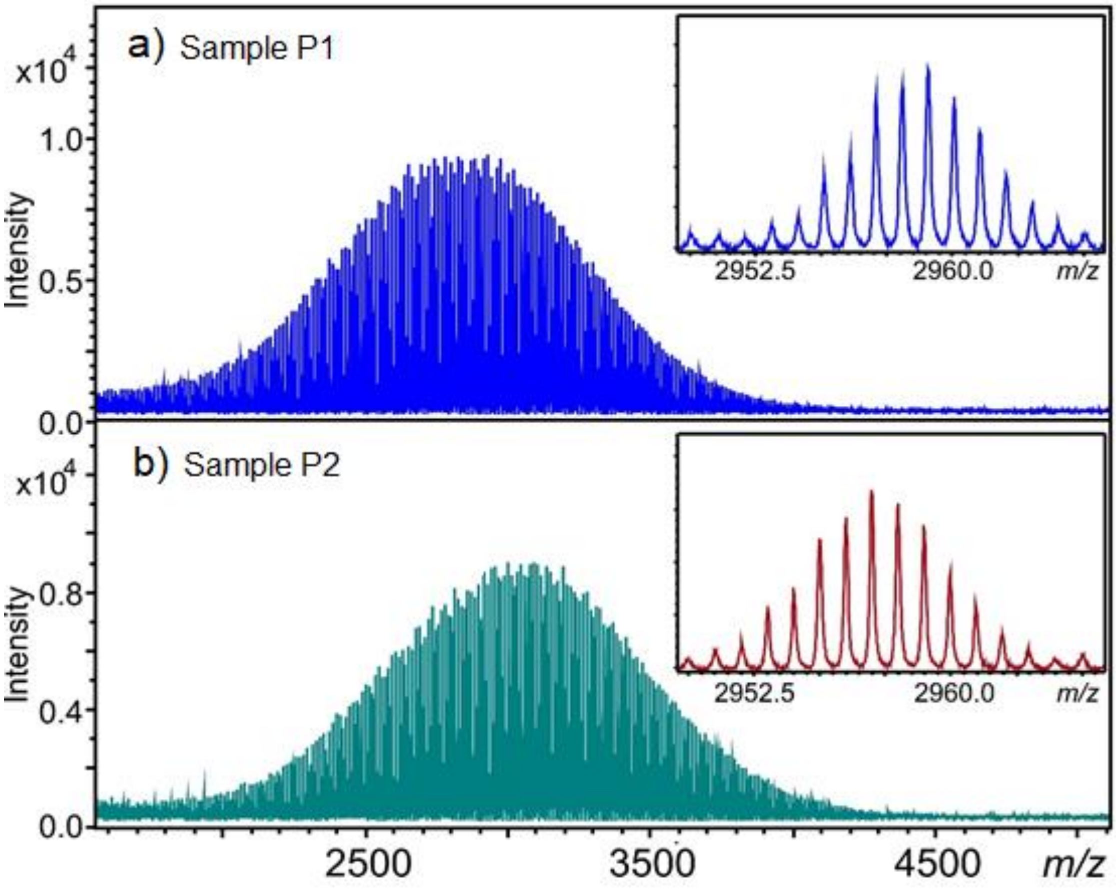
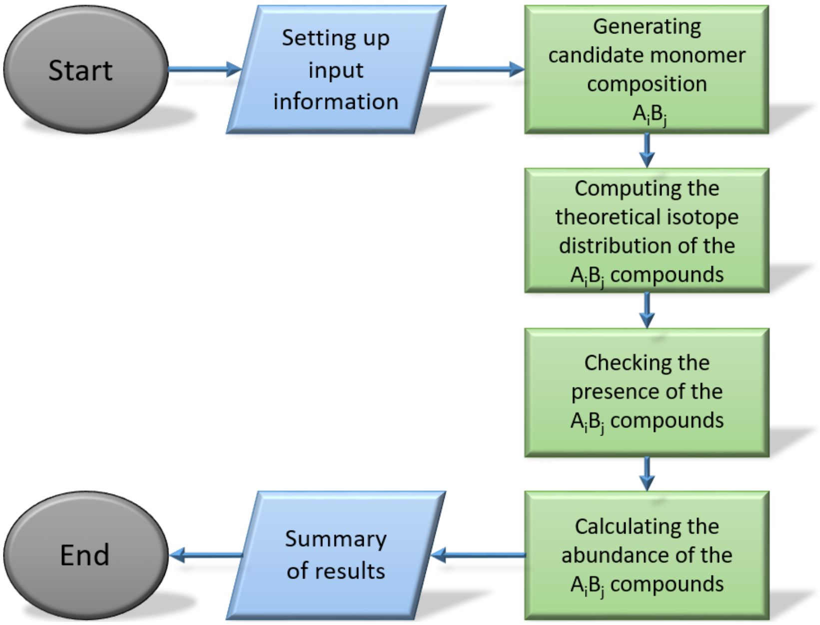


Figure 1. MALDI-TOF mass spectra of PEO–PPO–PEO copolymers with approximately 40 wt% EO content. (a) PE6400 from BASF (sample *P1*), (b) PE6400 analogue from Merck (sample *P2*).

As analogous copolymers with regard to composition and average mass (M_n ~ 2900), samples *P1* and *P2* have similar MALDI-TOF mass spectra as can be seen in Figure 1a and b. However, it is interesting as to whether the detailed evaluation of their mass spectra may reveal any differences in their chemical compositions and copolymer quantities. It would also be exciting to explore whether the occurring differences are reflected in the properties of these copolymers. Accordingly, our data processing approach will be introduced in the followings by presenting the detailed analysis for these two analogous poloxamer samples.



Scheme 1. Flow-chart of the algorithm for the compositional and relative abundance assignment.

The steps of our algorithm are as follows (see Scheme 1):

(1) Setting up input information. The copolymer mass spectrum is imported, normalized and stored in a relative abundance – *m/z* array. The exact masses of the end group, ionization agent and the monomers A and B are specified.

(2) Generating candidate monomer compositions A_iB_j. We tested two approaches: (i) The possible copolymer compositions are determined by solving the linear Diophantine equation $ax + by = c$ (using the extended Euclidean algorithm) for all the *m/z* peaks in the mass spectrum, where a and b are the nominal masses of the monomers and c is the transformed and converted integer value of an *m/z* peak minus the end group and the ionization agent. For example, taking the peak *m/z* 2956.99 in a PEO-PPO copolymer mass spectrum (see Figure 2, *Peak G*), the coefficients of the Diophantine equation are $a = 44$, $b = 58$ and $c = 2914$, and the two positive solutions of the $44x + 58y = 2914$ equation are 28; 29 and 57; 7. Based on the mass accuracy of our TOF analyzer, the latter can be excluded, thus the $(EO)_{28}(PO)_{29}$ composition is added to the *candidate monomer compositions* list. (ii) As a brute force approach, all the A_iB_j compositions are generated in the intervals $0 \leq i \leq i_{max}$ and $0 \leq j \leq j_{max}$, where i_{max}, and j_{max}, are determined based on the largest *m/z* value in the mass spectrum.

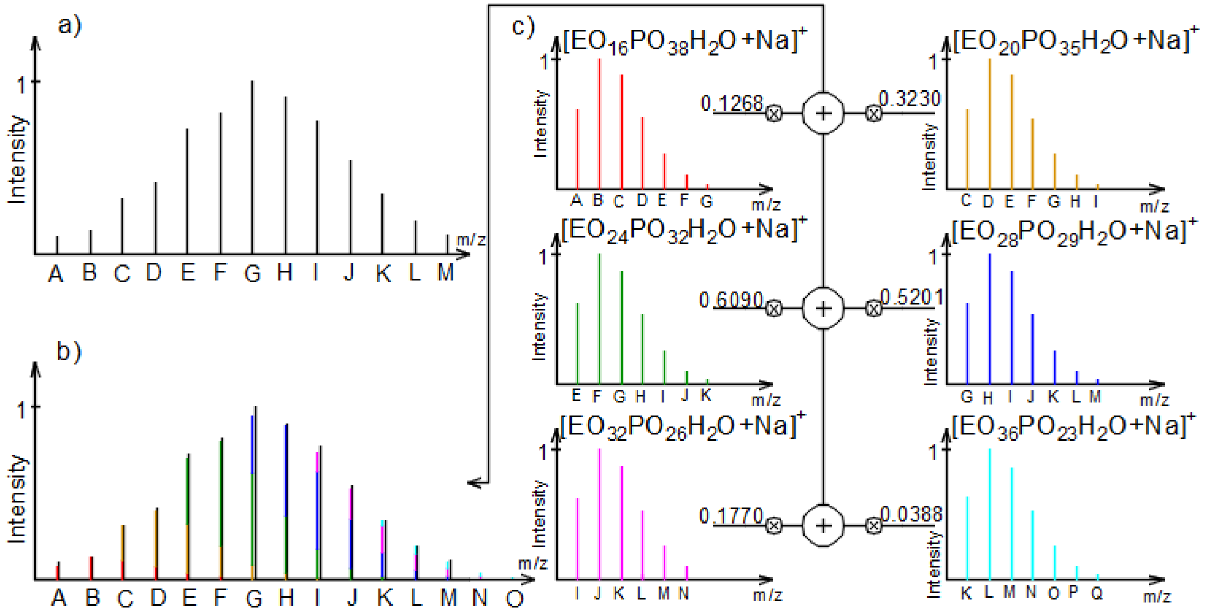
(3) Computing the theoretical isotope distribution of the A_iB_j compounds. The exact mass and abundance of 6-10 isotopomers (depending on the mass of A_iB_j) are calculated.

(4) Checking the presence of the A_iB_j compounds. An A_iB_j monomer composition is accepted as existing one in the mass spectrum if at least four peaks of its isotopic distribution have been found in the mass spectrum.

(5) Calculating the abundance of the A_iB_j compounds. The copolymer mass spectra consist of numerous peak clusters, each containing isotopic peaks, partially overlapped, of several A_iB_j compounds with a spacing of 1 g/mol. Here we introduce the R_{ij} coefficients for the abundance of each A_iB_j compound in this cluster. The intensity of a cluster peak is estimated by the sum $\sum_{i,j} R_{i,j}\, I_{i,j,k}$, where i,j are the indices of an A_iB_j compound contributing to the intensity of this

peak and I_{ijk} stands for the theoretical relative intensity of its k^{th} isotope ($k = 0$ denotes the monoisotopic peak). For instance, the *Peak G* in Figure 2 is estimated by the sum $R_{16,38} \cdot I_{16,38,6} + R_{20,35} \cdot I_{20,35,4} + R_{24,32} \cdot I_{24,32,2} + R_{28,29} \cdot I_{28,29,0}$. (It is specific for the ethylene oxide/propylene oxide copolymers that EO_xPO_y overlaps the second isotope peak of $EO_{x-4}PO_{y+3}$.) In order to find the R_{ij} coefficients, the distance between the estimated and measured cluster peaks were minimized by the least squares method. After the fitting procedure, the sum $R_{i,j} \sum_k I_{i,j,k}$ represents the total abundance of the composition A_iB_j, namely the sum of all of its isotopes. For example, the summarized abundance for the composition $EO_{28}PO_{29}$ is 0.5201 × (0.625 + 1.00 + 0.869 + 0.536 + 0.262 + 0.107 + 0.038).

The implementation of our algorithm is presented in the *DataProcessing.xlsm* spreadsheet in the Supporting Information.



	m/z		m/z		m/z		m/z		m/z		m/z
A	2951.02	D	2954.02	G	2956.99	J	2959.96	M	2962.96	P	2965.92
B	2952.03	E	2955.00	H	2957.98	K	2960.96	N	2963.94	Q	2966.92
C	2953.02	F	2955.99	I	2958.97	L	2961.95	O	2964.94		

Figure 2. Calculating the abundance of the EO_iPO_j compounds in a peak cluster. **a)** A representative peak cluster recorded by MALDI-TOF MS (see Figure 1b inset); **b)** theoretical isotope distributions of the peak cluster compounds; **c)** fitting on the experimental intensities.

The correct composition and abundance assignment enables the accurate calculation of the usual molecular weight averages M_n (number-average), M_w (weight-average), and the polydispersity index $PDI=M_w/M_n$. Moreover, additional quantities can be determined describing the chemical composition of the copolymer, such as the average molar fraction (c_A) and weight fraction (w_A) of unit *A* in the copolymer, the number-average number of units *A* and *B* (n_n^A, n_n^B), weight-average number of units *A* and *B* (n_w^A, n_w^B), and the polydispersity index for the monomers ($PDI_A=n_w^A/n_n^A$, $PDI_B=n_w^B/n_n^B$) [23]. Table 2 shows the values of these quantities for the two Pluronic PE6400 copolymers of different origin (samples *P1* and *P2*). Additional MALDI-TOF MS and NMR data for samples *P3-P9* will be presented and discussed later.

Table 2. Chemical composition of PE6400 from BASF (sample *P1*), and PE6400 analogue form Merck (sample *P2*) (PEO–PPO–PEO copolymers with approximately 40 wt% EO content) measured MALDI-TOF MS and NMR.

		sample *P1*	sample *P2*
MS	M_n	2706	2923
	M_w	2775	2979
	PDI	1.026	1.019
	c_{EO}	0.449	0.444
	w_{EO}	0.382	0.377
	n_n^{EO}	23.3	24.9
	n_w^{EO}	27.0	27.7
	PDI_{EO}	1.16	1.12
	n_n^{PO}	28.6	31.2
	n_w^{PO}	29.0	31.5
	PDI_{PO}	1.02	1.01
^{1}H NMR	M_n	2678	2830
	w_{EO}	0.405	0.391
	% of primary OH ends	85.4	84.9

As it turns out from the data of Table 2, remarkable differences can be recognized between the two Pluronic PE6400 analogues both in the weight averages and in the compositional properties. However, not only the average molecular weight and average copolymer composition can affect the properties of polymer but also the variation in the composition as the macromolecular chain length increases, i.e., the composition drift. The simplest way to visualize the composition drift is plotting the average molar fraction of one of the repeat units *versus* the degree of polymerization (i.e., the total number of EO and PO repeat units). Figure 3 depicts the composition drift plots of the two Pluronic PE6400 copolymers with different origin (samples *P1* and *P2*). Additional composition drift plots for the EO/PO triblock copolymers with various EO contents and number-average molecular weights (M_n) are given in Figures S1−S7 in the Supporting Information.

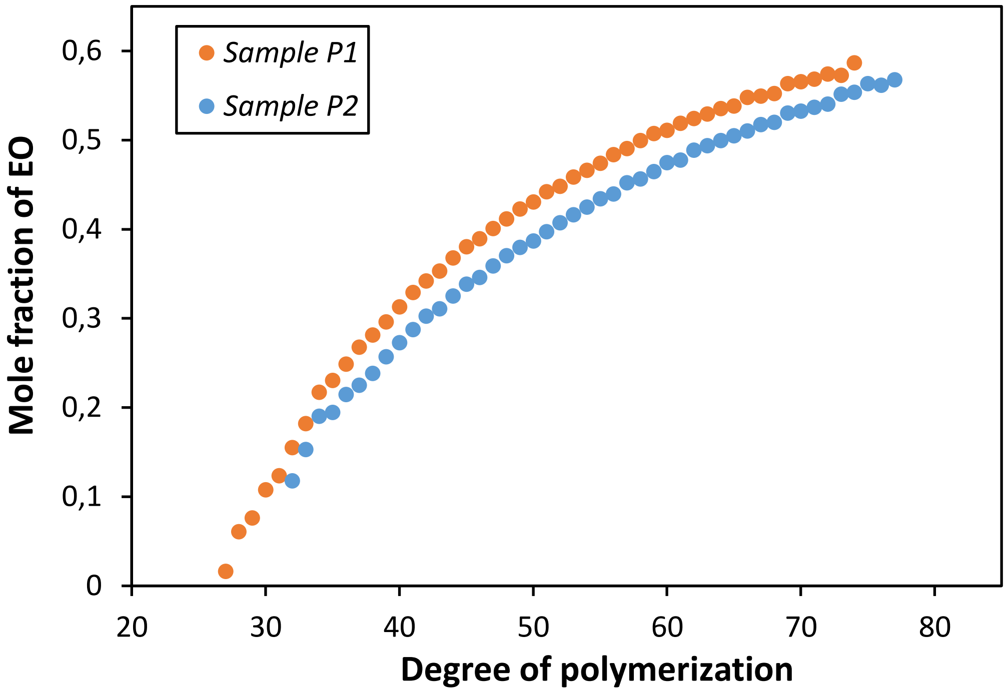


Figure 3. Composition drift plots for PE6400 from BASF (sample *P1*), and PE6400 analogue from Merck (sample *P2*) (PEO–PPO–PEO copolymers with approximately 40 wt% EO content). *Degree of polymerization* is the total number of EO and PO repeat units in the copolymer molecule.

As seen in Figure 3, a characteristic vertical shift can be observed that corresponds to the differences between the c_{EO} (and w_{EO}) average copolymer properties (see Table 2). The composition plots also indicate that the molar ratio of the EO unit increases with the degree of polymerization (where the latter is calculated as the total number of EO and PO repeat units in the copolymer chain). Thus, a question may arise as to what inference can be drawn from the shape of a drift plot, and how does it reflect the average composition parameters of the copolymer. Figure 4a and 4b shows the composition drift plots of samples *P1*, *P2*, and *P5* and the theoretically generated A_iB_j copolymer mass spectra. (The schematic of the algorithm for the simulation of A_iB_j copolymer mass spectra and an example are presented as Algorithm S1 and in *SimSpect.xlsm* in the Supporting Information.)

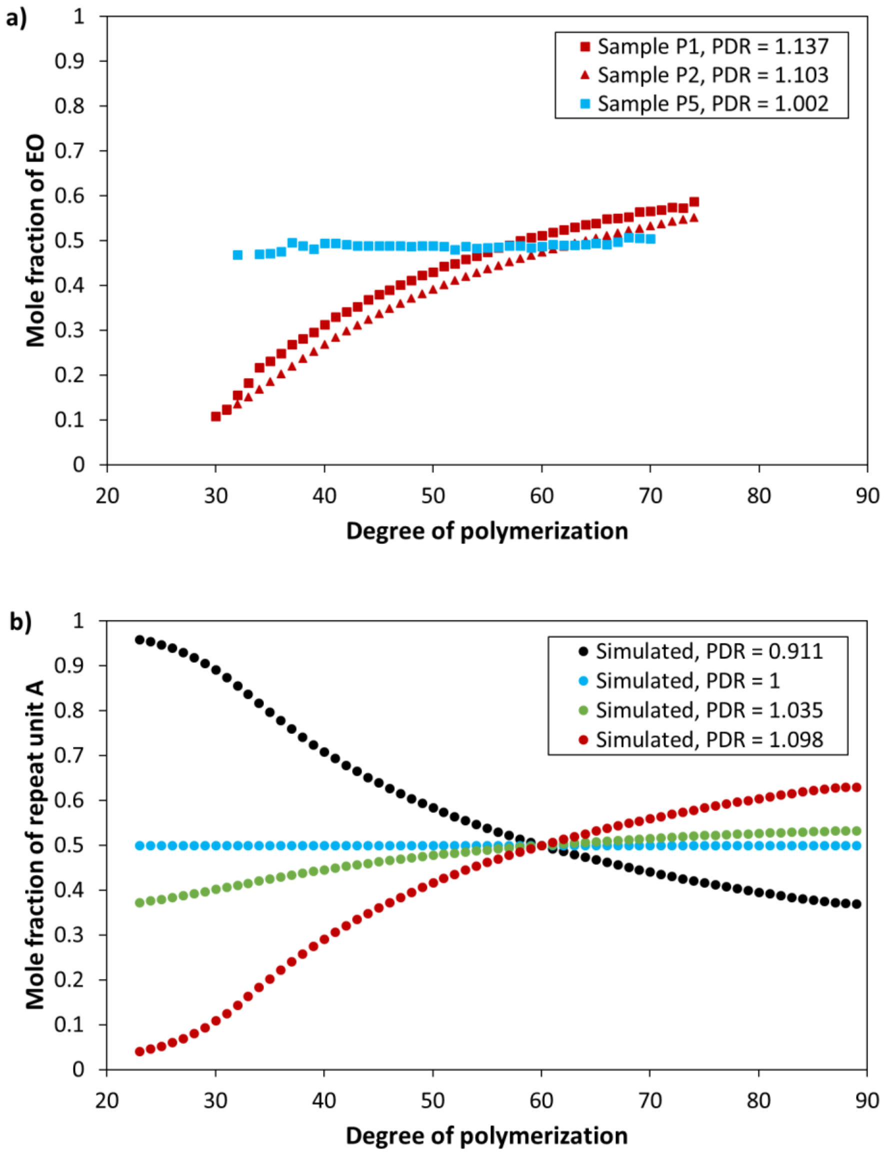


Figure 4. Composition drift plots for **a)** samples *P1*, *P2* and *P5* (see Figure S3) and **b)** theoretically generated A_iB_j copolymer mass spectra.

As seen in Figure 4, the governing parameter of the array of curves is the ratio of the polydispersity indices of the repeat units, thus we introduce a new copolymer quantity, the polydispersity ratio (PDR) of the comonomers as defined by Eq. 1.

$$PDR = \frac{PDI_A}{PDI_B} = \frac{n_w^A / n_n^A}{n_w^B / n_n^B} \qquad (1)$$

In the case of EO/PO block copolymers (Figure 4a), *PDR* is calculated as $PDR = PDI_{EO} / PDI_{PO}$ (see Table 2). As can be seen in Figure 4, if the repeat units have the same polydispersity, i.e., *PDR* is close to 1 (light blue and red horizontal plots in Figure 4a and Figure 4b, respectively), the relative amounts of the comonomers are constant throughout the entire molecular weight distribution, i.e., the composition drift is zero. In general, the difference $PDR - 1$ determines the slope of the composition drift plot. If $PDR > 1$, i.e., block *A* has a higher polydispersity value than that of block *B*, the drift curve shows a positive slope. On the contrary, if $PDR < 1$, the drift is negative. For all PEO-PPO-PEO triblock copolymers $PDR > 1$ was found, (i.e., the polydispersity value of the EO block is higher than that of the PO block), which indicates the presence of PEO-PPO diblocks (and/or even homopolymers) as impurities in the pluronic-type triblocks [25,26]. Indeed, the presence of diblocks was confirmed by NMR experiments for the PEO-PPO-PEO triblocks (see in Table 2 and in Figure S8, S9 in the Supporting Information), because secondary OH groups were detected supporting the lack of one PEO "arm" from the triblock. On the contrary, the horizontal composition drift plots (that suggest $PDR = 1$ values) of the reverse pluronic-type triblocks (i.e., PPO-PEO-PPO) (see Figure 4a and Figure S1-S5 in the Supporting Information) confirm that samples *P3-P5* are free from diblock copolymers. This finding is in line with those observed for the same reverse pluronics [27]. In addition, our NMR experiments revealed the presence of secondary OH groups for the reverse pluronic samples (Sample *P3-P7*, see Table 3), supporting the lack of PEO-PPO diblock impurities.

Thus, we believe that the shape and the run of the composition drift curve reflecting the PDR value offers a quick visual inspection for the detection of the presence of diblocks in a triblock copolymer product.

Furthermore, it can also be observed in Figure 4 that the experimental composition drift curves match well the simulated ones having approximately the same PDR values. It can be established that the composition drift plot carries a lot of information about the chemical composition of the copolymer such as: (i) the vertical position, shift expresses the average molar and weight fraction of unit *A* in the copolymer (as also seen in Figure 3), (ii) the horizontal position and width estimates the molecular weight distribution, and (iii) as it was discussed above, its slope is characteristic of the ratio of the polydispersity indices (PDR) of the blocks and indicates the presence or lack of diblock impurities. The composition drift plot can be especially useful when similar copolymers are compared by means of mass spectrometry.

In the followings, we will show how a relatively small difference in the composition drift curves of two PE6400-type Pluronics (see Figure 3) influence the copolymer properties. In order to study the aggregation behavior and micelle formation in aqueous solution, as one of the main application of Pluronics for drug delivery systems, dynamic light scattering experiments were performed.

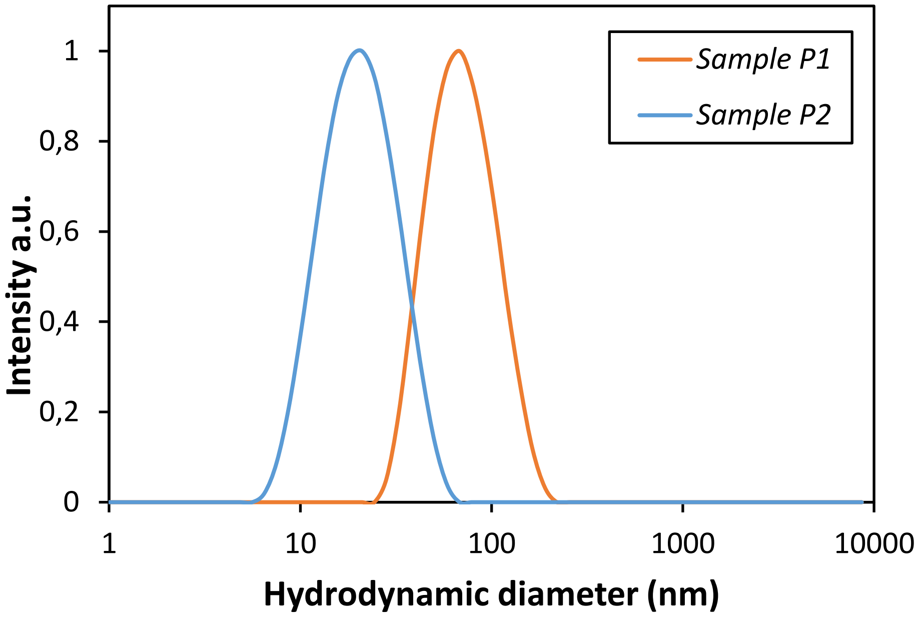


Figure 5. Dynamic light scattering (DLS) analysis of the PEO–PPO–PEO micelles formed in 3.45 mM (1 m/m%) aqueous solution. Intensity weighted size distribution of PE6400 from BASF (sample *P1*) and PE6400 analogue from Merck (sample *P2*).

Figure 5 shows the intensity weighted size distribution of the aggregates formed in the aqueous solution of two PE6400-type Pluronics (sample *P1* and sample *P2*). The difference is obvious, significantly smaller sized micelles were formed in the aqueous solution of sample *P2* than in the case of sample *P1* under the same experimental conditions. The deviation was statistically confirmed by 8-8 measurements of independent samples. The z-average hydrodynamic diameters (D_h) were determined to be 65.3 nm (RSD=3.7 %) and 18.2 nm (RSD=6.2 %) for sample *P1* and sample *P2*, respectively ($p < 10^{-5}$). Significant difference was also found with respect to the DLS polydispersity index (PDI_{DLS}), 0.114 and 0.206 for sample *P1* and sample *P2*, respectively ($p = 0.00004$).

The critical micelle concentration (CMC), in this case the concentration of block copolymers above which micelles form, is an important characteristic of the micellization process and can be controlled by small changes in the chemical structure of amphiphilic molecules or by varying the conditions of the disperse phase. Dynamic light scattering, together with various other techniques, enables the determination of the CMC [24]. Although DLS analyzes the temporal fluctuations in the intensity of scattered light in order to determine the diffusion coefficient and the particle size, however, as an additional feature of this technique, the CMC can also be determined by recording the concentration dependence of the time-averaged scattering intensity. Below the CMC, the intensity of the scattered light does not or only slightly depend on the concentration, and it has usually a very low value. However, once the CMC has been reached, due to the presence of micelles, the scattered light intensity increases approximately linearly with the concentration. This can be observed in Figure 6 that depicts the intensity of scattered light as a function of copolymer concentration for samples *P1* and *P2*. The concentrations and the corresponding scattered light intensities are compiled in Table S1 and S2 in the Supporting Information.

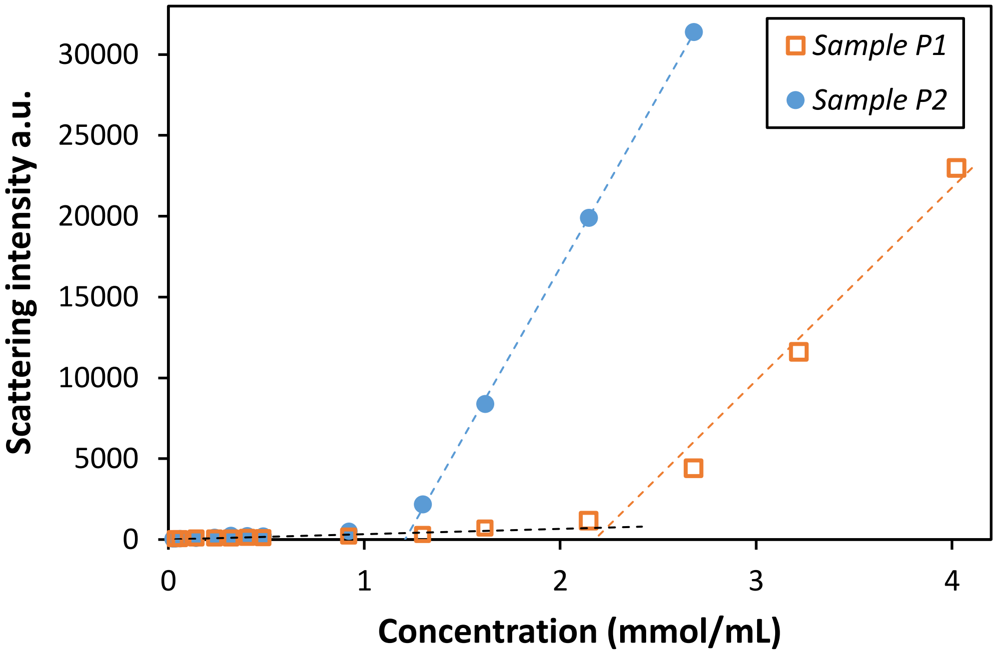


Figure 6. Plots of the average intensity of scattered light obtained for various concentrations of PE6400 from BASF (sample *P1*) and PE6400 analogue from Merck (sample *P2*) recorded by DLS.

As seen in Figure 6, two lines can be fitted on the scattered light *versus* concentration plots. The intersections between the two lines are at approximately 2.2 mM and 1.2 mM concentrations corresponding to the CMC of samples *P1* and *P2*, respectively. Similarly to the micelle size distribution obtained at a concentration well above the CMC (Figure 5), a significant difference can also be found in the CMC values of the two PE6400 Pluronics.

Table 3. Chemical composition of copolymer samples determined by MALDI-TOF mass spectrometry and NMR spectroscopy.

		sample *P3*	sample *P4*	sample *P5*	sample *P6*	sample *P7*	sample *P8*	sample *P9*
MS	M_n	2413	2412	2797	3394	3483	2763	2752
	M_w	2439	2437	2824	3427	3515	2809	2790
	PDI	1.011	1.011	1.009	1.010	1.009	1.017	1.014
	c_{EO}	0.503	0.506	0.486	0.197	0.219	0.131	0.144
	w_{EO}	0.434	0.437	0.418	0.157	0.175	0.103	0.113
	n_n^{EO}	23.6	23.8	26.4	12.1	13.8	6.4	7.0
	n_w^{EO}	24.1	24.2	27.0	12.8	14.3	9.2	9.7
	PDI_{EO}	1.02	1.02	1.02	1.06	1.04	1.43	1.37
	n_n^{PO}	23.3	23.2	27.8	49.0	49.2	42.4	41.7
	n_w^{PO}	23.8	23.6	28.4	49.5	49.7	43.0	42.2
	PDI_{PO}	1.02	1.02	1.02	1.01	1.01	1.01	1.01
^1H *NMR*	M_n	2396	2333	2754	3227	3388	2786	2760
	w_{EO}	0.434	0.429	0.415	0.140	0.173	0.135	0.136
	% of primary OH ends	0	0	0	0	0	47.4	53.5

Table 3 summarizes the detailed chemical compositions of sample *P3* to *P9* as determined by MALDI-TOF MS using our evaluation method and NMR spectroscopy. Samples *P3*, *P4*, *P5*, samples *P6*, *P7* and samples *P8*, *P9* are similar copolymers based on the available datasheets. The average molecular weights and the compositions of the copolymers are, however, different. For example, the number-average molecular weights of samples *P3*, *P4* and *P5* are 2413, 2412 and 2797, respectively. The copolymers from BASF are of lower average molecular weights than expected. Albeit, the average molecular weights of samples *P6* and *P7* are very similar, however, their EO contents are higher than those given in the manufacturer’s specification (0.1

w_{EO}). The observed deviations in the average molecular weights and compositions have been unambiguously supported by NMR measurements. The results of both methods are in line with each other validating the correctness of our evaluation method.

Conclusions

While there are several approaches to determine the average copolymer properties (e.g. M_n, M_w, *PDI*), it still requires special methods to describe the accurate chemical composition of the copolymer, especially to map the compositional drift. In this paper, we have proposed a robust algorithm for the compositional assignment and for the estimation of the relative abundance of all molecular species present in copolymer mass spectra. We demonstrated its effectiveness for the analysis of various PEO-PPO-PEO copolymers up to the average molecular weight of approximately 4000 g/mol. A huge benefit of our method is that it can easily be implemented in a common spreadsheet software and that it demands only low computing power and memory. An important output of our approach is the composition drift plot that can be especially useful when similar copolymers are compared by mass spectrometry analysis. The composition drift plot carries a lot of information about the chemical composition of the copolymer and, we found a characteristic relationship between the shape and run of the composition drift curve and the relative polydispersity indices (PDR, introduced in this paper) of the comonomers. This relationship can be used for the detection of diblock impurities in a triblock system. In order to confirm the copolymer quantities determined by our MS approach, additional ^{1}H NMR measurements were carried out. In each case, we found very good agreements between the results of the two methods. In addition, it was found that small differences in the chemical composition of copolymers of similar type considerably affect the copolymer properties, for example, the aggregation behavior of the amphiphilic block copolymers. This finding also emphasizes the importance of our new method (based on the composition drift curve and its relationship with the PDR value), because it can predict the presence of diblock impurities in a triblock copolymer that may have a remarkable influence on the properties and it can also be very useful in the quality check during the copolymer synthesis. In addition, our method can be extended to the indication of homopolymer impurities in a diblock copolymer.

ASSOCIATED CONTENT

Supporting Information

The following files are available free of charge.

Mass spectra, NMR spectra, DLS measurements (docx)

SimSpect (xlsm)

DataProcessing (xlsm)

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T.N., Á.K., M.Zs., S.K., Visualization; G.R., T.N., Á.K., Roles/Writing—original draft; G.R., T.N., Á.K., Writing—review &editing; M.Zs., S.K. All authors have read and agreed to the published version of the manuscript.

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The Polydispersity Ratio (PDR) and its Application for the Characterization of Poloxamers

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