

Characterization of polyacrylamide diblock copolymers by mass spectrometry combined with Mass-remainder analysis (MARA)

Tibor Nagy^a, Akos Kuki^a, Gergo Roth^{a,b}, Zuura Kaldybek Kyzy^a, Alifya Balqis Zatalini^a, David Nyul^{b,c}, Miklos Zsuga^a, Sandor Keki^{a,*}

^a Department of Applied Chemistry, Faculty of Science and Technology, University of Debrecen, Egyetem tér 1, H-4032, Debrecen, Hungary

^b Doctoral School of Chemistry, University of Debrecen, Egyetem tér 1, H-4032, Debrecen, Hungary

^c Department of Physical Chemistry, Faculty of Science and Technology, University of Debrecen, Egyetem tér 1, H-4032, Debrecen, Hungary

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ABSTRACT

Polyacrylamide block copolymers has been extensively used in many fields of science and technology due to their water solubility, biocompatibility and smart and tunable properties. In this study, we used the Mass-remainder analysis (MARA) and the Multi-step Mass-remainder analysis (M-MARA) for the processing of complex MALDI-TOF mass spectra of a series of poly(N-acryloylmorpholine)-block-poly(N-isopropylacrylamide) diblock copolymers. It was found that selection of the MARA base mass unit affects the shape of the Mass remainder *versus* m/z plots enabling to draw conclusions about the length and diversity of the blocks. Even better and more informative visualization can be achieved by creating 2-step M-MARA plots. The comprehensive characterization of the poly(N-acryloylmorpholine)-block-poly(N-isopropylacrylamide) copolymers with various block length were performed, and the results were confirmed by nuclear magnetic resonance (NMR) spectroscopy.

1. Introduction

Due to the water solubility, biocompatibility, stability, and ease of synthesis the acrylamide-based polymers have found various applications in many areas of science, technology and industry [1–3]. Furthermore, polyacrylamides (PAMs) can response to some environmental stimuli such as temperature, pH, solvent and redox processes, and their smart properties can be tailored by copolymerization of appropriate comonomers [4–6]. The smart behavior and the self-assembly of the PAM-based amphiphilic block copolymers in solution allows the creation of a variety of nanostructures that are frequently and widely used in drug delivery systems [7–9] as multifunctional chemosensors [10,11], nanoparticle stabilizers [12,13], shape memory [14], tissue engineering [15] and artificial muscles [16]. The chemical composition, molecular weight and macromolecular architecture can significantly affect the phase transitions and aggregation processes of these block copolymers [17]. Consequently, the development, synthesis and quality control in production of these advanced smart and functional materials require an accurate and comprehensive characterization of the copolymer structure. Mass spectrometry (MS) combined with matrix-assisted laser desorption/ionization (MALDI) [18,19] can be

effectively used to analyze copolymers [20–24] to obtain information on the individual polymer/copolymer chains. However, polymer molecules appear as isotopic peak clusters frequently yielding peak-rich mass spectra with thousands of m/z peaks. Furthermore, these peak clusters of copolymers are partially overlapped if the difference between the mass of the molecules with the monomer compositions A_xB_y and $A_x + nB_{y-m}$ (where A , B are the repeat units, n , m are small integers) is only a few (0, 1, 2, ...) mass unit [17]. Consequently, the evaluation of these mass spectra requires effective data processing methods and algorithms. The visualization methods can especially be useful, because they can reveal many important characteristics of the copolymers in a quick and easy way [25–27].

Recently, we have developed a mass spectrum visualization and analysis algorithm, called Mass-remainder analysis (MARA) and its extended version, the Multi-step Mass-remainder analysis (M-MARA) for the processing of complex mass spectra [25,27–30]. In this paper, we apply MARA, as a novel approach for the analysis of PAM-based amphiphilic block copolymers, particularly emphasizing its strength in the fast visual assessment of the main characteristics of copolymer composition.

* Corresponding Author.

E-mail address: keki.sandor@science.unideb.hu (S. Keki).

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2. Experimental

2.1. 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 *Trans*-2-[3-(4-*tert*-Butylphenyl)-2-methyl-2-propenylidene] malononitrile (DCTB) matrix and cesium chloride (CsCl) ionizing agent. Methanol was used as the solvent, and 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 ionizing agent solutions were mixed in the ratio of 5:2:1, respectively.

2.2. Nuclear magnetic resonance spectroscopy (NMR)

NMR measurements were carried out on a Bruker Avance II 400 MHz spectrometer equipped with a 5 mm z-gradient BBI probe. Quantitative 1D ^1H NMR experiments were performed at 295 K with a 45-degree 1H excitation pulse, a relaxation delay of 3 s, spectral width of 13.97 ppm, 32 k total data points, and 32 scans. Bruker TopSpin 4.0.9 software was used for NMR spectral processing and integration. For the NMR measurements, 6 mg of the sample was dissolved in 600 μL D_2O .

2.3. Chemicals

The N-acryloylmorpholine (NAM), N-Isopropylacrylamide (NIPA), 2,2'-Azobis(2-methylpropanamidine) dihydrochloride (initiator), 1,4-Dioxane, were obtained from Sigma Aldrich (Taufkirchen, Germany) and were used as received. 2-(Butylthiocarbonothioylthio)propanoic acid (CTA) was purchased from ABCR GmbH (Karlsruhe, Germany) and used without a purge. Water was purified by a Direct-Q water system (Millipore, Molsheim, France).

2.4. General procedure for the synthesis of block copolymers

Diblock copolymers by Reversible Addition/Fragmentation Chain Transfer (RAFT) polymerization were prepared based on the method described by Gody et al. [31,32]. For the first block, the chain transfer agent (CTA), monomer (NAM), initiator and the solvents with a magnetic stirrer were introduced into a test tube of 5 mL. The sealed tube was then placed in an oil bath thermostated at 70 $^\circ\text{C}$ to initiate the polymerization. After 20 min reaction time, the second reaction mixture was added (for the second block) by a syringe and the reaction was finished after another 20 min. The final volume of the reaction mixture was around 1 mL. At the end of the polymerization, the reaction mixture was extracted with diethyl-ether of 1 mL three times, then vacuum dried at room temperature.

The RAFT polymerization and the copolymer compositions were designed by the following equations.

$$DP_{\text{target A}} = \frac{[M_A]_0 P}{[CTA]_0 + 2f[I]_{1st} (1 - e^{-k_d t_1}) (1 - \frac{f_c}{2})} \quad (1)$$

$$DP_{\text{target B}} = \frac{[M_B]_0 P}{[LPC]_0 + 2f[I]_{2nd} (1 - e^{-k_d t_2}) (1 - \frac{f_c}{2})} \quad (2)$$

where the $[M_A]_0$, $[M_B]_0$, $[CTA]_0$ and $[I]_0$ are the initial concentrations of the A and B monomers, the chain transfer agent and the initiator, respectively. The k_d , t_1 and t_2 are the rate constant of the decomposition of initiator and the reaction time of the 1st and 2nd step, respectively. f is

the efficiency of the initiator, while the f_c is the coupling factor.

In order to implement the special RAFT polymerization method, preliminary experiments were carried out since the initiator used was different form that given in Refs. [31,32]. It was found that polymers only with the RAFT agent end were formed and the initiator decompose near completely within the reaction time under the applied reaction conditions. Furthermore, similarly to the given polymerization method we used 0.5 for the f factor and the coupling factor was 0. It must be noted, however, that the initiator has only a negligible effect on the molecular weight since the key to the controlled polymerization is the high chain transfer/initiator ratio. The name and the designed composition of the copolymers are shown in Table 1.

3. Results and discussion

In the following, we demonstrate the strength of the Mass-remainder analysis (MARA) with the characterization of diblock copolymers of N-acryloylmorpholine (NAM) and N-isopropylacrylamide (NIPA). The NAM- and NIPA-based temperature-responsive, water-soluble block copolymers are commonly used for various applications as detailed in the Introduction. A series of poly(NAM)-block-poly(NIPA) diblock copolymers with varying degree of polymerization were synthesized using reversible addition/fragmentation chain transfer (RAFT) polymerization as detailed in the Experimental and listed in Table 1. Fig. 1 shows the mass spectrum of the copolymer with the designed composition NAM₁₀NIPA₁₅ recorded by MALDI time-of-flight (TOF) MS. The samples for MALDI analyses were prepared using cesium ions as the cationizing agent in order to suppress the proton-cation exchange in the COOH end-group. In this way, a copolymer molecule with a given NAM_iNIPA_j composition is represented as a single peak in the mass spectrum (or more precisely as a single peak cluster of its isotopes) no other adduct ions, only the cesiated ones were detected.

The mass spectrum shown in Fig. 1a contains numerous peaks, approximately 2400. The large number of the peaks can be explained with the wide isotope distributions of the ions in this m/z range. For example, the ion corresponding to the composition NAM₁₂NIPA₁₇ has 8 isotopologues (above the 5% normalized intensity threshold) according to its theoretical isotope pattern. Moreover, the number of different NAM_iNIPA_j components cannot be derived directly from the total number of mass spectral peaks due to the overlapped isotope peak clusters as it will be detailed later. On the other hand, as it can be seen in the zoomed spectrum (Fig. 1b), the distances between the peaks can be assigned to the increments of the number of repeat units evidencing that the copolymer is composed of NAM and NIPA repeat units. The accuracy of the m/z values enables the assignation of the elemental composition and the determination of the end-groups. For example, the calculated and measured monoisotopic mass of the $[\text{NAM}_{12}\text{NIPA}_{17} + \text{Cs}]^+$ ($[\text{C}_{196}\text{H}_{333}\text{N}_{29}\text{O}_{45}\text{S}_3 + \text{Cs}]^+$) adduct ion is 4042.5 and 4042.3, respectively. The difference between the adjacent peaks (or peak clusters) is around 28 m/z unit, corresponding to the mass difference between the NAM and NIPA repeat units. In addition, as seen in Fig. 1b, a second series appears with minor intensities that can possibly be attributed to

Table 1

The name, number average molecular weight and composition of the designed copolymers.

sample name	$M_{n,calc}$	n_{calc}^{NAM}	n_{calc}^{NIPA}
S1	1509	5	5
S2	3486	15	10
S3	3911	10	20
S4	4051	15	15
S5	2780	10	10
S6	3346	10	15
S7	2215	10	5
S8	2920	15	5
S9	2640	5	15

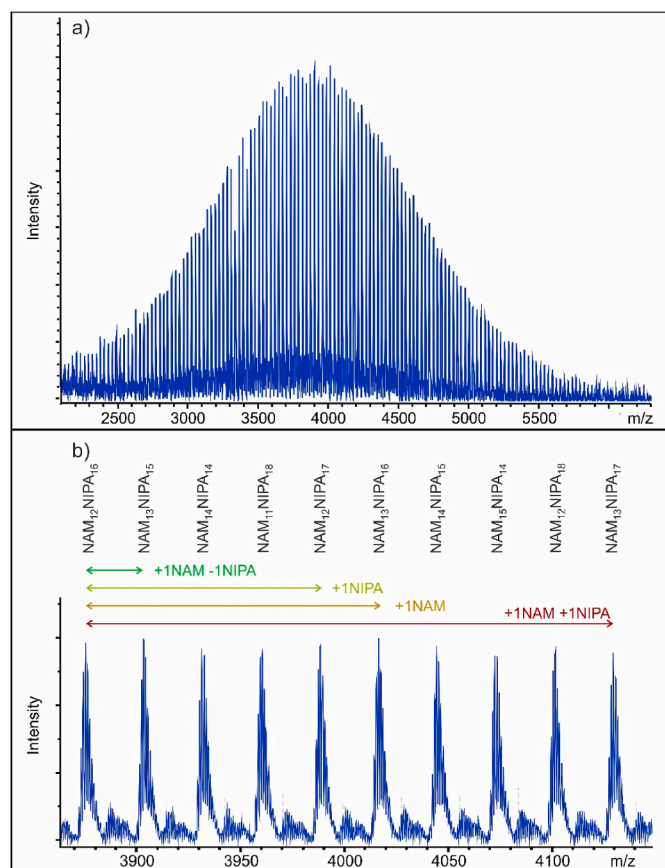


Fig. 1. The MALDI-TOF mass spectrum (a) and the zoomed spectrum of poly (NAM)-block-poly(NIPA) diblock copolymers with the designed composition $NAM_{10}NIPA_{15}$ (b) prepared with DCTB matrix and Cs^+ ionizing agent.

the irreversible chain termination leading to “dead chain” [33].

As we have shown, some characteristics of the copolymer sample can be determined by manual peak assignments, but as other complex chemical systems, the copolymers formed can be characterized by various distributions (e.g. molecular weight or block length distributions). This comprehensive analysis requires the assignment of hundreds or even thousands of m/z peaks, that is not feasible manually, thus using of software tools is preferable. Unfortunately, to the best of our knowledge, there is no such software available that can universally be used for the complete mass spectral characterization of copolymers due to their complexity and variety. However, we demonstrate how MARA, i.e., our data-mining method can be used for the sorting, grouping and simplifying such complex mass spectra. MARA simply performs a modulo (*MOD*) operation calculating the mass remainder MR_I after the division of the m/z value by a base mass unit R :

$$MR_I = m/z \text{ MOD } R.$$

(3)

(M-MARA repeats it twice or thrice.) The selection of the base unit (or units) is crucial in MARA in order to achieve effective sorting and grouping. MARA is also capable of revealing the possible overlaps in the copolymer mass spectra. It is essential for the correct calculations of the copolymer composition to handle the overlapped peaks as well [25]. In order to map the characteristics of the NAM/NIPA copolymer system, namely to identify the overlaps, a theoretical NAM/NIPA copolymer mass spectrum with only some peaks was subjected to MARA (see Fig. 2).

The exact mass of the NAM (i.e., $C_7H_{11}NO_2 = 141.0790$ Da) and NIPA (i.e., $C_6H_{11}NO = 113.0841$ Da) repeat units were chosen as the base unit (R in eq. (1)) to calculate $MR_I(NAM)$ (Fig. 2a) and $MR_I(NIPA)$ (Fig. 2b), respectively. The mass-remainder versus m/z plots (see Fig. 2) reveals homologous series differing only by the number of the base unit, as demonstrated by the horizontally aligned red dots in Fig. 2a corresponding to the NAM_xNIPA_y , $x = 0, 1, 2$, etc. composition. However, if we vary the number of NIPA units, different $MR_I(NAM)$ values are obtained (blue and green dots in Fig. 2a), and MARA uses these MR_I values for the determination of the number of NIPA repeat units and thereby finally the elemental composition as detailed in Ref. [25]. These are general features of MARA, but what are specific for the NAM/NIPA structure are the overlaps that can be demonstrated by the MARA plots. For example, as indicated in Fig. 2a, the red NAM_9NIPA_5 and blue NAM_5NIPA_{10} dots almost coincide with each other. The reason is that the replacement of 4 NAM by 5 NIPA units results only about 1 Da difference. It means that the first isotope peak of NAM_xNIPA_y overlaps the monoisotopic peak of $NAM_{x-4}NIPA_{y+5}$. We would like to stress once again that the correction of the overlapped mass peaks is essential for the true abundance calculation of the individual copolymer molecules.

In the following, we compare the $MR_I(NAM)$ and $MR_I(NIPA)$ plots of copolymer samples with various average block length. Fig. 3 shows the entire and zoomed MR_I versus m/z plots of the copolymer with NAM: NIPA 15:5 designed average block length (see Table 1, sample S8).

As seen in Fig. 3a and b, effective sorting was achieved by MARA using both NAM and NIPA as the base mass unit, the dots corresponding to the peaks in the mass spectrum are grouped in 5 and 4 groups, respectively. In Fig. 3c, the line at $MR_I = 32.8$ corresponds to the m/z peaks of the NAM_xNIPA_2 copolymer species. The line above (Fig. 3a), at $MR_I = 33.9$ represents the first isotopes of NAM_xNIPA_2 components possibly overlapped with the peaks of $NAM_{x-4}NIPA_7$. The third line at $MR_I = 35.1$ might be composed of the isotopes of the first and second line and the $NAM_{x-8}NIPA_{12}$ monoisotopes, and so forth. But, if we compare the relative intensity (represented by the dot diameter) of the peaks aligned vertically in Fig. 3c, it is very similar to the theoretical isotope distribution of the $NAM_{17}NIPA_2$ copolymer. It suggests, that the overlaps of the $NAM_{x-4}NIPA_7$, $NAM_{x-8}NIPA_{12}$, etc. can be neglected, thus the dots of the zoomed $MR_I(NAM)$ plot correspond to the “clean” NAM_xNIPA_2 copolymer species. This is also supported by the unimodal intensity distribution of the rows in Fig. 3c. Thus, we can conclude that the copolymer sample under study contains various and relatively long NAM block and short NIPA blocks, that is in line with the designed NAM:

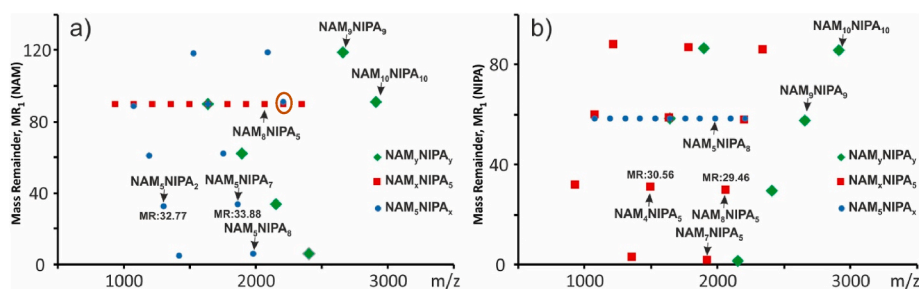


Fig. 2. The MR_I versus m/z plots of a theoretical mass spectrum with some calculated NAM/NIPA copolymer peaks using the exact mass of a, NAM and b, NIPA as the R base mass unit. ($x = 0-10$, $y = 5-10$).

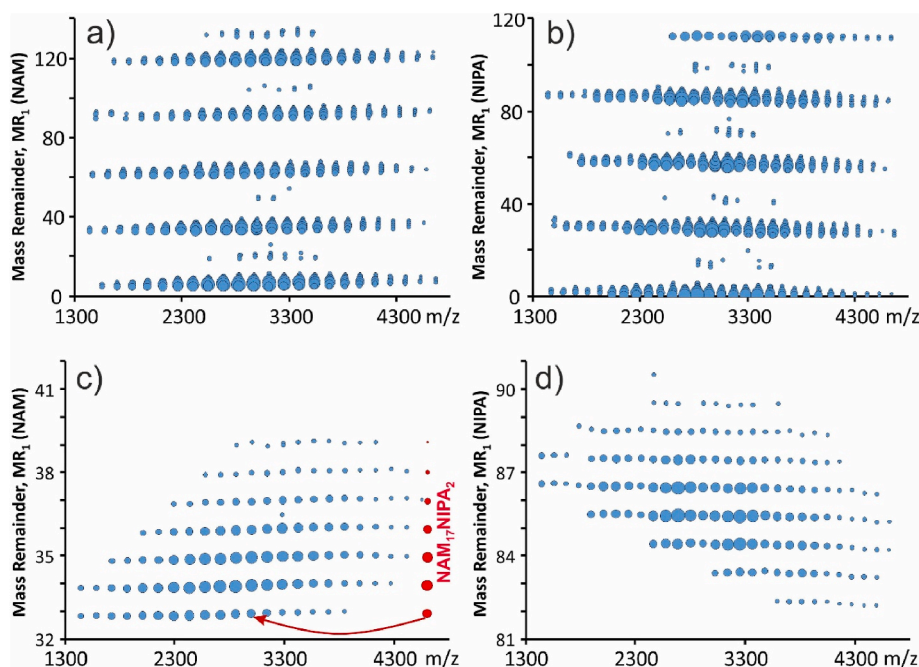


Fig. 3. The MR_1 versus m/z plots of the copolymer with NAM:NIPA 15:5 average block length (Sample S8) using the exact mass of NAM (a,c) and NIPA (b,d) as the base mass unit. c and d zoom in one-one group (horizontal line) of the graphs above. The dot diameters indicate the relative intensity of the m/z peaks.

NIPA 15:5 average block length. This conclusion is more evident, if we compare the MR_1 (NAM) and MR_1 (NIPA) plots. In Fig. 3d, the rows have multimodal intensity distribution, indicating that the dots and rows are composed of the overlapped isotope peaks of the copolymer species having NAM_n , NAM_{n+4} , NAM_{n+8} , etc. blocks. For instance, the MR_1 (NIPA) values of the $NAM_{10}NIPAY$, $NAM_{14}NIPAY$, $NAM_{18}NIPAY$, and $NAM_{22}NIPAY$ compounds are 85.4, 84.3, 83.2, and 82.1, respectively. The “steps” in the figure (five NIPA base unit to the right, one mass unit to the bottom) indicate also the appearance of the +4 NAM block increment moving towards the higher m/z regions (cf. the mass of 4 NAM units equals that of 5 NIPA units – 1 Da). Summarizing the observations presented above, the MARA can reveal the possible overlaps of the m/z peaks and provide a quick visual evaluation of the main characteristics of the NAM/NIPA copolymers, but it must be noted that the accurate quantitative analysis requires the intensity correction of the overlapped peaks.

In the previous paragraph we analyzed one-one group of the MR_1 (NAM) and MR_1 (NIPA) plots merely (that are zoomed in Fig. 3c and d). However, it is necessary to evaluate all the groups shown in Fig. 3a and b in order to get an overall view of the copolymer characteristics and draw more reliable conclusions. This general analysis can be facilitated by the application of the Multi-step Mass-remainder analysis (M-MARA). As seen in Fig. 3a and b, the groups in the MARA plots are shifted

vertically to each other by $MR_1 = 27.995$ that equals to the mass difference of the NAM and NIPA repeat units and corresponds to the mass of the CO group. This mass difference is used as the divisor R_2 in the second MARA step:

$$MR_2 = MR_1 \text{ MOD } R_2 \quad (4)$$

The MR_2 (NAM, CO) and MR_2 (NIPA, CO) versus m/z plots are shown in Fig. 4a and b.

As seen in Fig. 4, M-MARA eliminates the repetitive pattern of the MARA plots (shown in Fig. 3a and b) their groups are merged. We can recognize that the point clouds in the M-MARA plots have similar shape than the single zoomed groups of the MARA plots (Fig. 3c and d), so we can justify the conclusion, which was drawn above, namely, the copolymer sample contains various and relatively long NAM blocks and short NIPA blocks. Fig. 5 shows the M-MARA plots of the copolymer sample with NAM:NIPA 5:15 average block length (Sample S9), to make it more clear how the MR_2 (NAM, CO) and MR_2 (NIPA, CO) versus m/z plot pair can inform us on the main characteristics of the copolymer.

As seen in Fig. 5a, the MR_2 (NAM, CO) plot has a tilted, stretched shape with steps similar to the MR_2 (NIPA, CO) plot in Fig. 4b. It suggests that the sample S9 contains various and relatively long NIPA blocks and short NAM blocks on the contrary to sample S8 (see Fig. 4). It is in line with the designed NAM:NIPA 5:15 average block length.

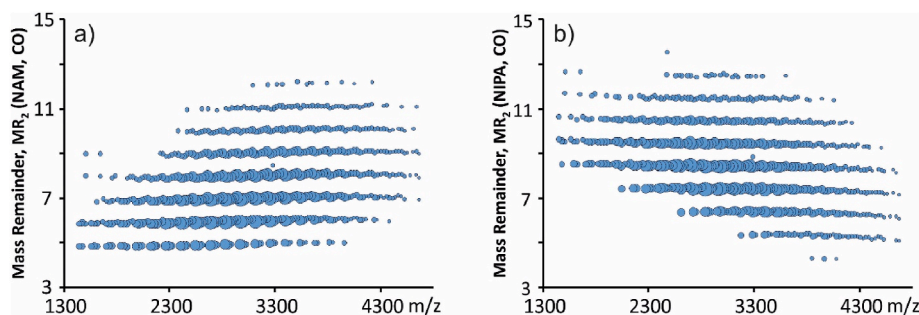


Fig. 4. The MR_2 (NAM, CO) versus m/z (a) and MR_2 (NIPA, CO) versus m/z plots (b) of the copolymer with NAM:NIPA 15:5 average block length (Sample S8). (The MR_2 (NIPA, CO) versus m/z plot is shifted up by 6 units for better visualization.).

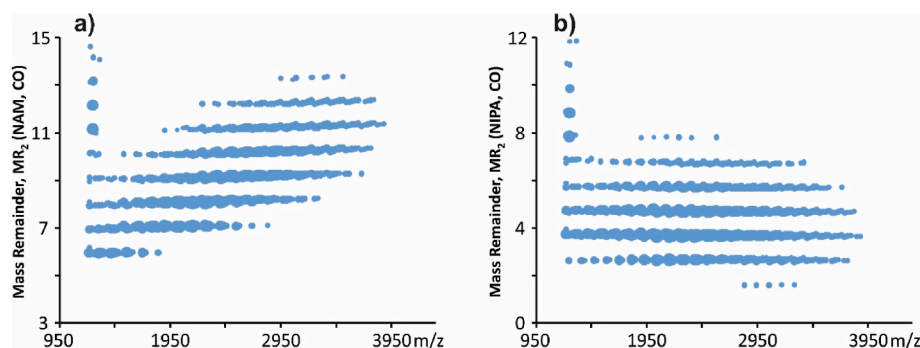


Fig. 5. The $MR_2(\text{NAM}, \text{CO})$ versus m/z (a) and $MR_2(\text{NIPA}, \text{CO})$ versus m/z plots (b) of the copolymer with NAM:NIPA 5:15 average block length (Sample S9). (The $MR_2(\text{NIPA}, \text{CO})$ versus m/z plot is shifted up by 6 units for better visualization.).

So far, we have created the MARA and M-MARA plots using both the NAM and NIPA repeat unit as the base mass unit. The comparison of the M-MARA plots as demonstrated in Figs. 4 and 5 may determine, which base unit yield more effective sorting and should be used for further analysis, such as for the assignment of the m/z peaks, deisotoping, overlapped peak correction, and so on [25]. In the case of sample S8 the choice of NAM resulted in better sorting (Fig. 4a), but for sample S9, NIPA should be chosen as the base unit (Fig. 5b).

In the M-MARA, the MR_2 versus MR_1 plot can also be created that gives another useful visualization and representation of the copolymer mass spectra (Fig. 6).

If the first base unit is NAM, the position of a dot in the MR_2 versus MR_1 plots depends on the number of NIPA units as indicated in Fig. 6. The MR_2 versus MR_1 plots facilitate the recognition and identification of the characteristic differences and repetitions (see the arrows in Fig. 6) of the mass spectrum peaks, thereby helping the interpretation and evaluation of the copolymer mass spectra. Since MARA does not require any transformation to a new mass scale, the MR_1 and MR_2 differences in Fig. 6 correspond to the m/z differences between the mass peaks.

After the main characteristics of the NAM/NIPA mass spectra were explored by MARA (e.g. overlaps, repetitions), we corrected the intensities of the overlapped peaks and then summarized the intensities of all the isotope peaks belonging to the same $\text{NAM}_x\text{NIPA}_y$ copolymer specie. It was performed by our algorithm detailed in Ref. [17]. The accurate calculation of the relative abundance of the copolymer species enables the reliable and comprehensive characterization of the NAM/NIPA copolymer samples with various designed chain length. Table 2 summarizes the usual molecular weight averages and many additional quantities describing the chemical composition of the copolymers.

To confirm our results obtained by MALDI-TOF MS and M-MARA, nuclear magnetic resonance (NMR) spectroscopy experiments were

performed. The ^1H NMR spectrum together with the assignments for sample 8 is shown in Fig. 7.

The number-average molecular weight (M_n) of the copolymers can be determined by ^1H NMR, since the signal of the methyl group at the chain-end of the chain transfer agent appears below 1 ppm. In addition, the NAM and NIPA repeat units can unambiguously be identified. Above 5 ppm, the presence of hydrogen atoms next to the double bond of the unreacted monomers can be detected. It should be noted that their integrated signal is small, i.e., approximately total conversion was achieved. The copolymer properties determined by ^1H NMR are also listed in Table 2. As it turns out from the data of Table 2, the values obtained by the two methods, in most cases, are in good agreement. Furthermore, the measured values are also in line with the designed copolymer parameters given in Table 1. This finding indicates that the 2,2'-Azobis(2-methylpropionamide) dihydrochloride initiator can be adopted for the RAFT polymerization of the NAM and NIPA monomers. The detailed MS analysis (see Table 2) enables the deeper insight into the polymerization process. The polydispersity indices of the poly(NAM) blocks are around 1.1, but a correlation can be observed between the length and polydispersity of the poly(NAM) blocks with a Pearson's r value -0.80 . The same tendency can be seen for the poly(NIPA) block with $r = -0.88$. However, it is to be noted, that homopolymers were not detected in the copolymer samples, thus the broader block length distribution is not due to the coexistence of the homo- and copolymers.

4. Conclusions

Mass-remainder analysis (MARA) and its extended version, the Multi-step Mass-remainder analysis (M-MARA) was effectively used for processing of the mass spectra of a series of poly(N-acryloylmorpholine)-block-poly(N-isopropylacrylamide) diblock copolymers. The Mass remainder (MR_1) versus m/z and the MR_1 versus MR_2 plots enhanced the interpretation and evaluation of the copolymer mass spectra by the recognition and identification of the characteristic differences and repetitions of the mass spectrum peaks. The MARA plots can easily be created even by a spreadsheet software. They give a visual assessment of the mass spectra and provide a quick tool to draw conclusions on the block length and length distribution of the acrylamide based block copolymers. This simplicity and speed can especially be favorable in the quality control of copolymer synthesis and manufacture. After the visual evaluation, an accurate and comprehensive characterization of the various NAM/NIPA diblock copolymers were performed. Furthermore, the MALDI-MS – MARA results were supported by NMR experiments. The visual tools of MARA and the subsequent spectrum processing algorithms can be adopted for the analysis of different kind of acrylamides and other copolymers, too.

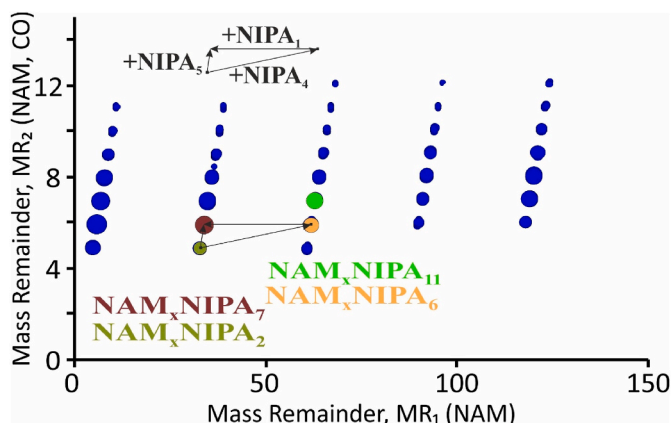


Fig. 6. The $MR_2(\text{NAM}, \text{CO})$ versus $MR_1(\text{NAM})$ plot of sample S8.

Table 2

Chemical composition of the poly(N-acryloylmorpholine)-block-poly(N-isopropylacrylamide) diblock copolymer samples determined by MALDI-TOF MS – M-MARA and ^1H NMR.

		S1	S2	S3	S4	S5	S6	S7	S8	S9
MS	M_n	1612	3787	3762	3810	2804	3290	2385	2895	2390
	M_w	1720	3912	3883	3946	2966	3434	2529	3030	2577
	PDI	1.067	1.033	1.032	1.036	1.058	1.044	1.060	1.046	1.078
	c_{NAM}	0.525	0.586	0.257	0.436	0.352	0.333	0.742	0.797	0.219
	w_{NAM}	0.579	0.638	0.301	0.491	0.404	0.384	0.782	0.830	0.259
	η_{NAM}^{NAM}	5.6	16.0	7.5	12.4	7.3	8.3	11.9	15.6	3.9
	η_w^{NAM}	6.6	17.1	8.2	13.9	8.6	9.2	12.9	16.6	5.1
	PDI_{NAM}	1.18	1.06	1.08	1.12	1.16	1.11	1.09	1.06	1.29
	η_{NIPA}^{NIPA}	5.1	11.3	21.8	16.1	13.5	16.6	4.1	4.0	14.1
	η_w^{NIPA}	6.0	12.2	22.7	17.6	14.7	17.8	5.1	5.2	15.4
	PDI_{NIPA}	1.18	1.07	1.04	1.09	1.09	1.07	1.23	1.31	1.09
^1H NMR	M_n	1825	3680	3552	3759	2898	3130	2421	2897	2680
	η_{NAM}^{NAM}	6.9	17.3	8.5	14.2	9.8	9.3	12.2	15.9	5.4
	η_{NIPA}^{NIPA}	5.4	8.9	18.7	13.4	11.3	14.0	4.0	3.6	14.9
	c_{NAM}	0.560	0.661	0.312	0.516	0.463	0.400	0.752	0.814	0.264

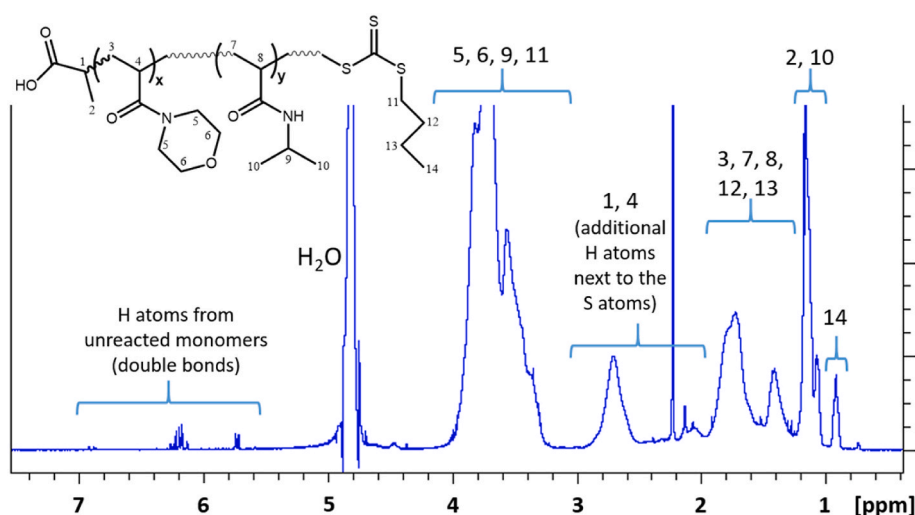


Fig. 7. The ^1H NMR spectrum of sample 8 recorded in D_2O .

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Author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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