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Coupling of NMR and Liquid Chromatography at Critical Conditions: A New Tool for the Block Length and Microstructure Analysis of Block Copolymers

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ABSTRACT: The online coupling of liquid chromatography at critical conditions (LCCC) and NMR was used for the analysis of block copolymers. Polyisoprene-*b*-poly(methyl methacrylate) (PI-*b*-PMMA) copolymers synthesized by living anionic polymerization were separated regarding chemical composition by LCCC using single solvents as mobile phases. The analysis of the chemically different LCCC fractions was conducted by on-flow ^{1}H NMR. To separate the copolymers from the respective homopolymers, critical conditions of polyisoprene (PI) and poly(methyl methacrylate) (PMMA) were used. Critical conditions were obtained by varying the column temperature. The comprehensive chemical composition and microstructure analysis by ^{1}H NMR revealed that quantitative information on the PI/PMMA content as well as the microstructure of PMMA (isotactic, atactic, and syndiotactic triads) and PI (1,2-PI, 1,4-PI, 3,4-PI) was obtained. For the first time the determination of the true molar masses and true chemical compositions of the block copolymers was achieved by determining the microstructure distribution.

Introduction

Block copolymers are extremely important polymeric materials due to the fact that large molecular segments of different polarities, solubilities, or crystallization behavior are combined in one molecular structure. In solid state they form multiphase morphologies. Block copolymers are traditionally used as compatibilizers for polymer blends. They are also suitable stabilizers of micro- and nanoemulsions. Block copolymers are of increasing interest for application as drug delivery systems, as organic semiconductors, and in fuel cells.[1]

The unique properties of block copolymers are determined not only by the total molar mass and the lengths of the different blocks but also by the microstructure of each compositional unit and the presence of homopolymers of each monomer type. Therefore, it is of major interest to optimize the synthetic procedures for block copolymers to ensure that the desired molecular structure is obtained and the amount of byproduct is minimized. Controlled radical polymerization (CRP)[2] and living anionic or cationic polymerizations[3,4] are the most frequently used techniques for synthesizing diblock and triblock copolymers. However, the detailed characterization of the copolymers is still a challenge. In particular, the precise determination of the true block lengths is not straightforward. Very specific chromatographic separation methods have to be used for this aim. It is the goal of this paper to introduce a new tool for the determination of the true molar masses of block copolymers based on the entire microstructure analysis of the block copolymers.

Liquid chromatography at the critical point of adsorption (LCCC) is a powerful tool to separate macromolecules. It was described by Entelis and Gorshkov[5,6] and Tennikov and Belenkii.[7−9] This method is based on the concept that for a given homopolymer chromatographic conditions can be found where all molecules irrespective of their chain length coelute in one chromatographic peak. This regime is achieved by compensation of the enthalpic and entropic interactions between the macromolecules and the chromatographic system (stationary and mobile phase). The polymer at critical conditions is also called "chromatographically invisible".[10−12] This concept was introduced by Gorbunov and Skvortsov.[10] It indicates that the polymer does not contribute to retention.

The coupling of LCCC with NMR is especially useful for the analysis of macromolecules. After Berek et al.[13] demonstrated that LCCC can be used to separate poly(methyl methacrylate) regarding tacticity, Kitayama et al.[14] analyzed poly(ethyl methacrylate) according to the tactic triads by LCCC-NMR. In this case, the NMR detector allowed the quantification of the microstructure distribution. Further applications of LCCC-NMR are presented in refs 15 and 16, where mixtures of poly(ethylene oxides) could be separated and analyzed regarding their different end groups.

LCCC is also a very important method for the analysis of block copolymers which can be used to separate the polymers selectively with regard to the lengths of the different blocks. Zimina et al. reported the first applications on block copolymers.[17,18] Several further applications of LCCC of block copolymers have been published. For example, Pasch et al. analyzed PS-*b*-PMMA[19−21] and block copolymers of poly(decyl methacrylate)-*b*-poly(methyl methacrylate).[22] Falkenhagen et al. used LCCC for the analysis of PMMA-*b*-poly(*tert*-butyl methacrylate).[23] Lee et al. used this method to analyze PS-*b*-PI.[24] Baran et al. characterized polystyrene-*b*-poly(ethylene oxide) copolymers.[25] These papers have used

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Table 1. PMMA and 1,4-PI Homopolymers of Different Molar Masses and Their Microstructures Determined by ^{1}H NMR[a]

PMMA			polyisoprene		
M_w (kg/mol)	M_n (kg/mol)	mm/mr/rr (mol %)	M_w (kg/mol)	M_n (kg/mol)	1,4/3,4 (mol %)
4.2	3.9	1.9/29.1/69.0	2.6	2.3	90.5/9.5
11.4	10.4	1.8/25.1/73.1	4.8	4.5	94.8/5.2
22.7	21.6	1.4/21.5/77.1	8.4	7.9	94.7/5.3
79.7	77.1	1.5/21.7/76.8	9.8	9.2	94.5/5.5
133.6	120.4	2.4/24.5/73.1	20	19.3	94.7/5.3
166.4	162.1	1.2/18.5/80.3	32.2	30.9	95.1/4.9
230.0	221.0	1.4/20.3/78.3	45.9	44.5	95.1/4.9
491.5	475.2	2.4/24.1/73.5	94.4	91.1	95.1/4.9

[a] mm = isotactic triad, mr = atactic triad, and rr = syndiotactic triad of α-CH_3 of PMMA.

solvent mixtures to achieve critical conditions. As Zimina et al.[18] have shown, the use of solvent mixtures can cause broadening of the LC peaks. Berek et al. confirmed this behavior for homopolymers.[26] Another approach to achieve critical conditions of block copolymers was reported by Chang et al.[27] He used a single solvent as the mobile phase to separate PS-*b*-PI. Macko et al.[28] used the same concept to obtain critical conditions of several other homopolymers.

In block copolymer analysis, LCCC can be used to separate the copolymer from homopolymer fractions of the different blocks. At the same time conditions can be found where one block of e.g. a diblock copolymer is at critical conditions while the other block elutes in a size exclusion mode (SEC). The size distribution of this block can be determined via standard SEC calibration. This kind of block length calibration via homopolymers was also studied by Wang et al.[29] They found differences of the partition coefficients of homopolymers and copolymers especially if the first block of the copolymer is shorter than the other. Differences of the molar masses of diblock and triblock copolymers versus homopolymers were also observed by Chang.[30]

We have recently shown that the coupling of LCCC and ^{1}H NMR provides quantitative information on the molar mass distribution (MMD) and the chemical composition distribution (CCD) of polystyrene−poly(methyl methacrylate) block copolymers by using solvent mixtures.[31,32]

Now we will demonstrate the power of LCCC-NMR for the analysis of block copolymers of PI-*b*-PMMA. These diblock copolymers consist of a polyisoprene (PI) and a poly(methyl methacrylate) (PMMA) block connected by a covalent linkage. They are expected to contain the respective homopolymers as byproduct. This new method provides the true molar mass distribution and the true chemical composition distribution of the block copolymers as well as their microstructures. Specifically, the detailed analysis of the microstructure is new and important for the comprehensive analysis of the block copolymers. As compared to complementary chromatographic methods using solvent mixtures and standard concentration detectors, the new method of LCCC-NMR is faster and reveals more structural details.

NMR is by far the most powerful detection method that can be used in liquid chromatography of polymers. However, when directly coupled to SEC or HPLC, the intrinsically low sensitivity and the effect of the mobile phase on the NMR measurement have to be addressed. Typically, LCCC is conducted in protonated binary mobile phases. The proton signals of the mobile phase are a major concern in on-flow LCCC-NMR since they may cover regions in the spectrum that are vital for detecting the polymer proton signals. In the present contribution, critical conditions for both blocks of the block copolymers are obtained by using only single solvents as mobile phases. This simplifies the spectra by significantly reducing the number of unwanted solvent signals. The remaining solvent signals will be efficiently suppressed by a suitable pulse sequence (WET).[33]

Experimental Section

Synthesis of the Block Copolymers. The PI-*b*-PMMA block copolymers were synthesized by living anionic polymerization with PI as the first polymerized block[34] using *sec*-butyllithium as the initiator. 1,4-PI and 3,4-PI configurations were formed. The reactions were conducted in a typical apparatus for anionic polymerizations that was directly attached to a vacuum line for reactant purification and handling. All solvents and monomers were purified by destillation and drying. They were kept under argon to exclude moisture and oxygen. After the formation of the block copolymers the solvents were removed from the reaction mixtures, the polymers were dissolved in dichloromethane and extracted with water. After extraction the polymer in the dichloromethane solution was precipitated in petrol ether. The amounts of initiator and monomers were chosen with respect to the chemical compositions and total molar masses that were targeted.

1,2-PI-*b*-PMMA. 300 mL of purified THF (Sigma-Aldrich) was given into the reactor, mixed with the initiator (0.5 mL of *sec*-butyllithium (1.4 M)), and cooled to −80 °C. Using maximum stirring, cold purified isoprene (20.7 g = 30.4 mL (0.304 mol)) was added to the initiator solution as fast as possible and reacted for 30 min. The reaction mixture was brought to room temperature. Samples were taken at regular intervals and precipitated in methanol, and the molar mass was analyzed by SEC (molar mass 30 kg mol^{-1}). After a 100% consumption of polyisoprene was obtained, diphenylethylene (0.117 mL = 6.6×10^{-4} mol) was added to the reaction mixture and stirred for 20 min. The solution changed the color to red. Before the second monomer, methyl methacrylate was added, the reaction mixture was cooled again to −80 °C. 9.3 g (9.94 mL) of cold MMA was added at maximum stirring. After a reaction time of roughly 30 min the living block copolymer was quenched with methanol.

1,4-PI-*b*-PMMA. Purified cyclohexane (456 g, VWR HiPerSolv Chromanorm) was given into an ampule and mixed with the purified isoprene (61.29 g = 0.8997 mol, Acros). To this monomer solution the initiator (4.3 mL of *sec*-butyllithium (1.4 M)) was added. The reaction mixture was shaken 16 h at room temperature. A sample was taken and precipitated in methanol, and the molar mass was analyzed by SEC (molar mass 10 kg mol^{-1}). Diphenylethylene (0.79 mL = 0.004 45 mol) was added to the reaction mixture and shaken overnight at room temperature (red color of the solution).

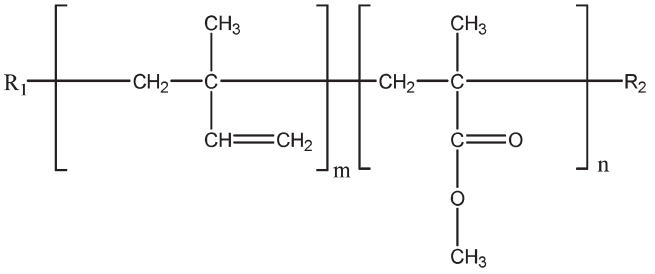
Purified THF (300 mL) was given in a reactor, and the living polyisoprene (125 mL) was added. Afterward, the reaction mixture was cooled to −80 °C, and 7 mL (0.065 mol) of cold destilled MMA (Fluka) added under vigorous stirring. After a reaction time of roughly 30 min the living block copolymer was quenched with methanol.

For calibration 1,4-PI homopolymers and syndiotactic PMMA homopolymers of different molar masses are used (see Table 1). These samples were produced by PSS GmbH Mainz, Germany.

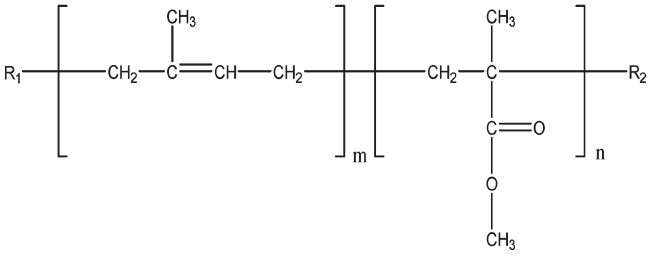
HPLC. The HPLC system was an Agilent model 1100. It consists of a pump, a UV detector, a column oven, and an ELSD 1000 detector from Polymer Laboratories-Varian. The column

Scheme 1. Structure of PI-*b*-PMMA Copolymers with (a) 1,2-PI, (b) 1,4-PI, and (c) 3,4-PI Units

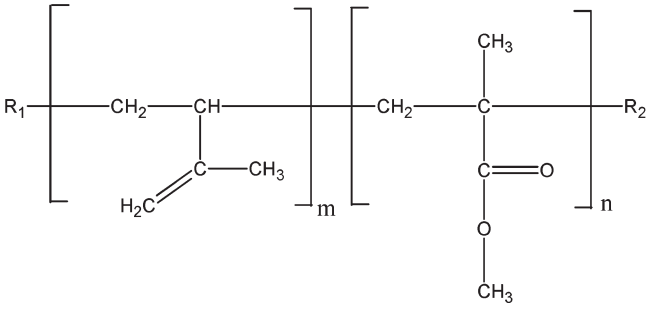
a) 1,2 PI-b-PMMA



b) 1,4 PI-b-PMMA



c) 3,4 PI-b-PMMA



oven can regulate the temperature with an accuracy of 0.1 °C. The HPLC separations were carried out on Nucleosil Si 300-5 + Si 1000-7 (Macherey-Nagel) with column sizes of 200 × 4.6 mm i.d. as well as Nucleosil C_{18} 300-5 + C_{18} 1000-7 (Macherey-Nagel) with column sizes of 250 × 4 mm i.d. The concentration of the injected samples was 0.5 mg/mL. 10 μL was injected. The flow rate was 0.5 mL/min.

HPLC-NMR. The HPLC-NMR experiments were performed with an AVANCE-400 NMR spectrometer (Bruker BioSpin GmbH) attached to the Agilent HPLC system 1100 via a loop collector interface. The NMR experiments were carried out with an inverse triple resonance flow probe equipped with a pulsed field gradient coil. The flow cell has an active volume of 60 μL. The ^{1}H 90° pulse was 4.7 μs. The LCCC-NMR experiments were performed with a concentration of 10 mg/mL for the homopolymers of the blends and 30 mg/mL for the block copolymers. 50 μL was injected. The flow rate was 0.5 mL/min.

On-flow HPLC-NMR experiments were carried out by using protonated HPLC solvents. WET solvent suppression was applied to the HPLC solvents. Eight scans per FID with 16 kB data and 1.1 s repetition delay were acquired. The series of FIDs were Fourier transformed via one time domain and plotted as 2D contour plots of retention times vs chemical shifts.

Table 2. PI-*b*-PMMA Copolymers of Different Block Lengths Estimated by SEC and the Average Chemical Composition of PI and PMMA Determined by ^{1}H NMR

	PI-*b*-PMMA		
sample	M_w (kg/mol)	M_n (kg/mol)	PI/PMMA (mol %)
1	18.8	17.7	91.4/8.6
2	19.3	18.3	50.7/49.3
3	90.2	85.7	15.9/84.1
4	102.6	99.8	25.4/74.6
5	52.0	45.0	55.6/44.4

Results and Discussion

PI-*b*-PMMA copolymers (Scheme 1) of different PMMA block lengths were synthesized. Two different types of block copolymers were produced containing predominantly either 1,4-PI or 3,4-PI blocks. The block copolymer that was selected for the present investigations to demonstrate the power of LCCC-NMR consisted predominantly of 3,4-PI units with smaller amounts of 1,4- and 1,2-PI units. This is sample 5 of Table 2 which has a nominal molar mass of M_w = 52 kg/mol, M_n = 45 kg/mol (determined by size exclusion chromatography, SEC), and an average chemical composition of 55.6:44.4 mol % of isoprene to methyl methacrylate units (determined by ^{1}H NMR).

Typical LCCC applications are based on using interactive stationary phases and binary solvent combinations as mobile phases to adjust the enthalpic and entropic interactions in the system. Alternatively, these interactions can be adjusted by varying the column temperature.

Critical conditions for PI and PMMA are determined by measuring homopolymers of different molar masses on stationary phases of different polarities. This stationary phase screening has to be conducted with different mobile phases at different temperatures. Preferably, the stationary phase is selected such that one homopolymer (e.g., PI) is at the critical point of adsorption and the other homopolymer (e.g., PMMA) elutes in the SEC mode.

Critical Conditions of PMMA. Critical conditions of PMMA are found on a set of polar stationary phases Si 300-5 + Si 1000-7 (column sizes of 200 × 4.6 mm i.d.) and ethyl acetate as mobile phase. Si indicates nonmodified polar silica gel. The critical conditions of PMMA were obtained by adjusting the column temperature to 10 °C. Figure 1 shows the diagram for establishing critical conditions. At the critical point of adsorption, all PMMA standards are eluting at the same elution volume. Critical conditions are characterized by the fact that the PMMA elution is independent of the molar mass. On the other hand, PI is eluting in SEC mode as indicated by the dotted line in Figure 1. For temperatures above 10 °C the PMMA standards elute in the adsorption mode (LAC). It is unusual that the LAC mode is reached with increasing temperatures. However, this phenomenon can be caused by an entropy increase and was discussed by Lochmüller et al.[35] and Chang et al.[36,37] This unusual behavior and a possible influence of the end groups of low molar mass polymers might also cause the slightly earlier elution for smaller molar masses in the LAC mode (crossing of the calibration curves at low molar masses). The PMMA standards exhibit the same microstructure as the copolymers (about 70−80% syndiotactic as shown in Table 1). Therefore, the LCCC conditions obtained with the PMMA standards are applicable to the analysis of the block copolymers.

Critical Conditions of PI. The critical conditions of PI are obtained by using a set of nonpolar stationary phases C_{18} 300-5 + C_{18} 1000-7 (column sizes of 250 × 4 mm i.d.). The annotations stand for C_{18}-alkyl bonded porous silica gel with average pore sizes of 300 and 1000 Å. The nonpolar

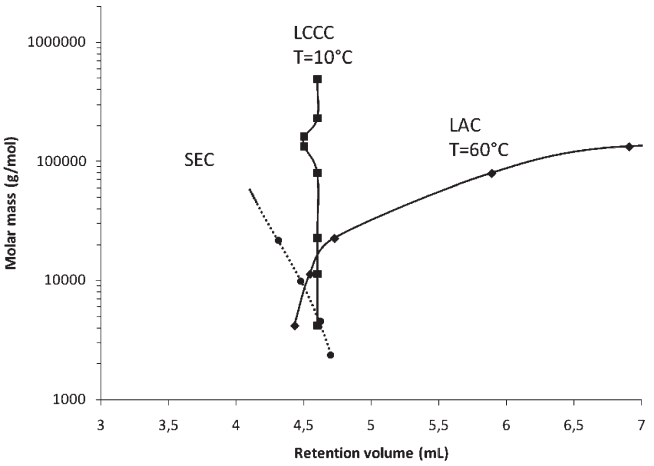


Figure 1. Critical diagram of molar mass M vs retention volume for PMMA, stationary phase: Nucleosil Si 300-5 + Si 1000-7, mobile phase: ethyl acetate, flow rate: 0.5 mL/min, detector: ELSD; column temperature: (■) T = 10 °C and (◆) T = 60 °C; (●) and dotted line = PI molar mass calibration with LCCC-NMR at critical conditions of PMMA (SEC = size exclusion chromatograpy, LCCC = liquid chromatography at critical conditions, LAC = liquid adsorption chromatography).

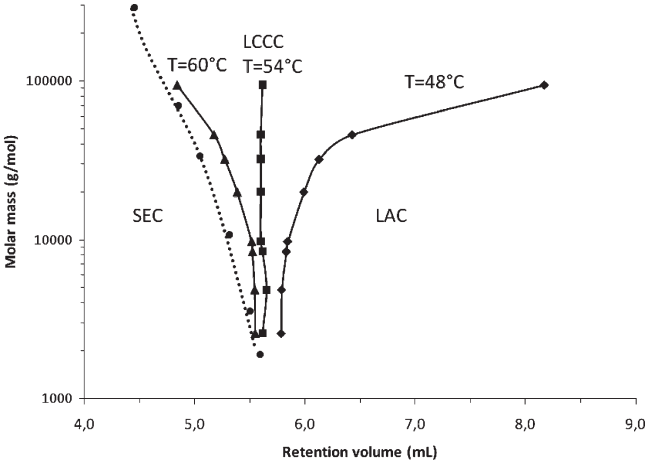


Figure 2. Critical diagram of molar mass M vs retention volume for PI, stationary phase: Nucleosil C_{18} 300-5 + C_{18} 1000-7, mobile phase: 1,4-dioxane, flow rate: 0.5 mL/min, detector: ELSD, column temperature: (▲) T = 60 °C, (■) T = 54 °C, (◆) T = 48 °C; (●) and dotted line = PMMA molar mass calibration with LCCC-NMR at critical conditions of PI.

stationary phase is selected because the polarity of PI is lower than the polarity of PMMA. Accordingly, PI exhibits stronger enthalpic interactions with the stationary phase than PMMA. Dioxane was used as the mobile phase.

Figure 2 shows the behavior of different molar mass standards of PI vs elution volume. The critical conditions for PI are adjusted by varying the column temperature. It can be seen from Figure 2 that critical conditions are obtained exactly at a temperature of 54 °C. In agreement with the expected LCCC behavior, all PI samples elute at the same elution volume irrespective of their molar mass. PMMA is eluting in SEC mode under these conditions.

Both critical conditions could be obtained by varying the temperature with an accuracy of 1 °C. It should also be noted that the column dimensions were suitable to achieve a sufficient SEC separation.

Please note that the SEC calibration curves in Figures 1 and 2 are presented only for demonstration purposes. They

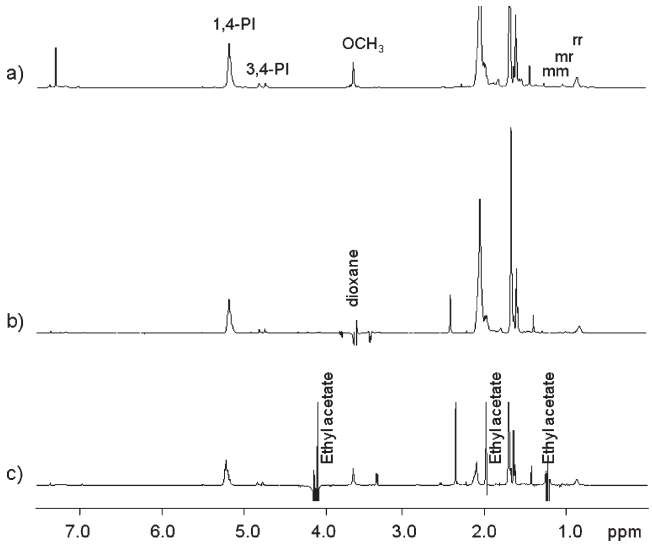


Figure 3. ^{1}H NMR spectra of PI-*b*-PMMA (sample 1): (a) in $CDCl_3$, (b) in dioxane, and (c) in ethyl acetate (solvent signals of dioxane and ethyl acetate were suppressed with WET).

were obtained by SEC-NMR while the critical condition measurements were obtained using an ELS detector. Therefore, the elution volumes obtained by both methods are not completely comparable (different capillary lengths, different detector cell volumes).

Analysis of the PI-*b*-PMMA Sample. When selecting specific solvents as mobile phases, the requirements of the chromatographic and NMR experimental conditions have to be considered. Regarding NMR, it is important to ensure the detection of the relevant polymer signals. Figure 3 shows ^{1}H NMR spectra of sample 1 in ethyl acetate, dioxane, and chloroform-*d*. Dioxane and ethyl acetate require solvent suppression. Only one resonance of dioxane and three resonances of ethyl acetate are suppressed. In case of dioxane the olefinic PI and the isotactic (mm), atactic (mr), and syndiotactic (rr) triads of the α-CH_3 group of PMMA can be detected. The OCH_3 group of PMMA overlaps with dioxane. In the case of ethyl acetate the signals of the olefinic PI, OCH_3, and the atactic (mr) and syndiotactic (rr) triads of the α-CH_3 group are visible. WET solvent suppression was applied. The same technique was also used for the on-flow LCCC-NMR experiments.

The following results are obtained by LCCC-NMR for PI−PMMA blends and the block copolymer samples:

(1) Two selected blends of PI and PMMA (PI (M_w = 20.0 kg/mol) + PMMA (M_w = 22.7 kg/mol) and PI (M_w = 94.4 kg/mol) + PMMA (M_w = 22.7 kg/mol)) were prepared and analyzed to evaluate the chromatographic separation under both critical conditions.

It is evident from Figure 4 that blends of the homopolymers PI and PMMA can be well separated by LCCC-NMR independently of the chosen critical conditions. Figure 4a shows an on-flow experiment at critical conditions of PMMA where PI is eluting in SEC mode. Figure 4b illustrates the LCCC-NMR experiment at critical conditions of PI and the SEC mode of PMMA. Both diagrams show a clear separation of the two components.

The molar masses of the homopolymers eluting in SEC mode are in good agreement with the calibration curves shown in Figures 1 and 2. It should be noted that the retention volumes of the critical conditions in Figures 1 and 2 were obtained with an ELS detector. They are different from those in Figure 4a,b. The LCCC-NMR experiments

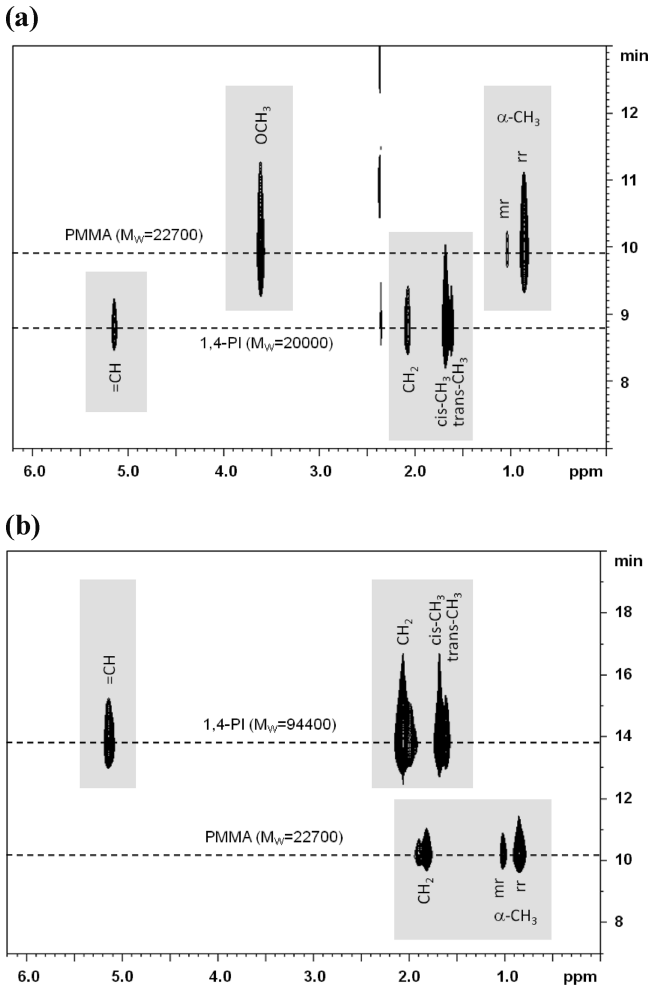


Figure 4. (a) LCCC-NMR of a blend of 1,4-PI (M_w = 20.0 kg/mol) and PMMA (M_w = 22.7 kg/mol) at critical conditions of PMMA (ethyl acetate at column temperature T = 10 °C) (10 mg/mL for each polymer, 50 μL injection volume, 0.5 mL/min flow rate). (b) LCCC-NMR of a blend of 1,4-PI (M_w = 94.4 kg/mol) and PMMA (M_w = 22.7 kg/mol) at critical conditions of PI (1,4-dioxane at column temperature T = 54 °C) (10 mg/mL for each polymer, 50 μL injection volume, 0.5 mL/min flow rate) (dashed lines = peak maxima).

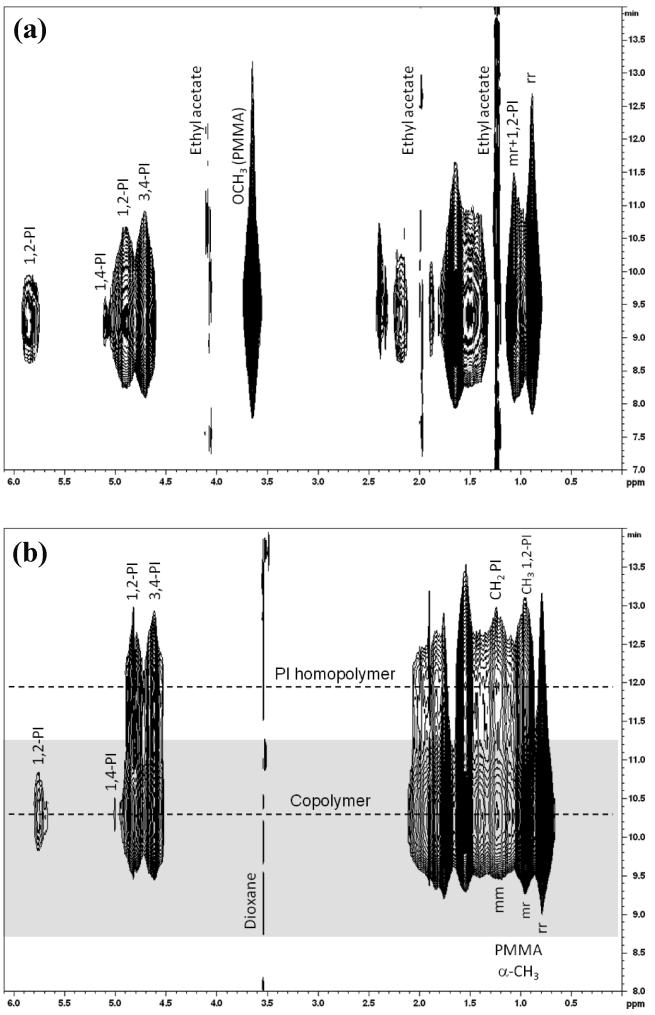


Figure 5. (a) LCCC-NMR on-flow run of the PI-*b*-PMMA copolymer sample 5 (M_w = 52 kg/mol) at critical conditions of PMMA (30 mg/mL of copolymer, 50 μL injection volume, 0.5 mL/min flow rate). (b) LCCC-NMR on-flow run of the PI-*b*-PMMA copolymer sample 5 (M_w = 52 kg/mol) at critical conditions of PI (30 mg/mL of copolymer, 50 μL injection volume, 0.5 mL/min flow rate).

provide larger retention volumes at critical conditions due to the larger NMR flow cell and longer capillaries resulting in additional delays. As a consequence, Figures 1 and 2 show calibration curves (determined by SEC-NMR) which cross the curves of critical conditions recorded by the ELSD.

(2) The application of a single solvent as the mobile phase and ^{1}H NMR as the concentration detector allows the independent detection of both monomer units. As a consequence, it is even possible to detect and quantify the microstructures of both monomer units at any given retention time. Thus, 1,2-, 1,4-, and 3,4-PI sequences and isotactic (mm), atactic (mr), and syndiotactic (rr) PMMA sequences can be measured with high precision.

(3) The study of block copolymers with LCCC-NMR allows the separation of block copolymers and the corresponding homopolymers. Critical conditions can be chosen such as that one polymer block experiences critical conditions while the other elutes in SEC mode. Figure 5 shows the on-flow LCCC-NMR experiments of the block copolymer (sample 5) at both critical conditions. The olefinic regions of the 1,2-, 1,4-, and 3,4-PI sequences as well as the tacticity of the α-CH_3 group of PMMA are well resolved in both cases. Whereas measurements at critical conditions of PI only need the solvent suppression of one signal (mobile phase is dioxane), critical conditions of PMMA require solvent suppression of three frequencies (mobile phase is ethyl acetate). Dioxane overlaps with the OCH_3 signal of PMMA; however, the α-CH_3 group is visible. The critical conditions of PI are very useful for separating PI homopolymer from the block copolymer. The peak maxima of the copolymer and homopolymer regions are indicated by dashed lines in Figure 5b. Using ethyl acetate as the mobile phase at critical conditions of PMMA, full information on the OCH_3 group and the olefinic region is obtained, but partial overlapping of a 1,2-isoprene signal (CH_3) with the α-CH_3 group (mr triad) of PMMA is observed (see Figure 5a).

In any case, it is possible to calculate the chemical composition of the copolymer at any given retention time for both critical conditions.

(4) The chemical composition of both blocks can only be correctly determined by separating the true copolymer from precursor homopolymers. This is achieved at critical conditions of PI for separating PI-*b*-PMMA from homopolymer PI. Figure 5b shows the separated regions for the homopolymer (white area) and the copolymer (gray area). The determination of the CCD requires the precise quantification of the microstructures of both monomer units at each retention

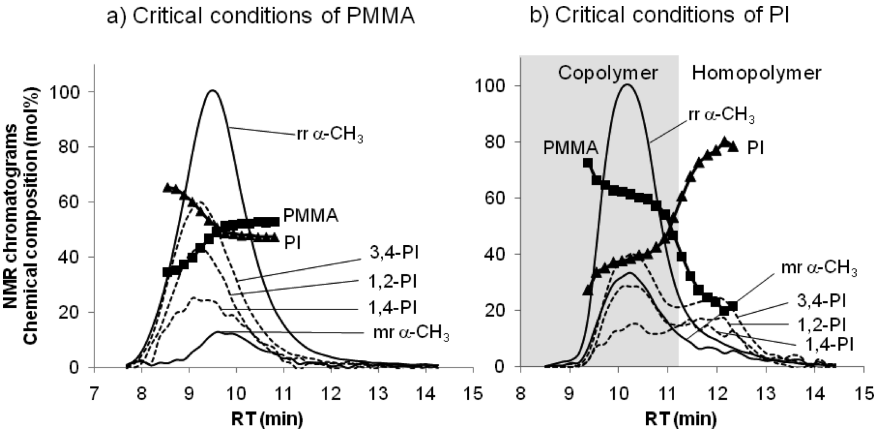


Figure 6. Chemical composition of the 3,4-PI-*b*-PMMA copolymer (sample 5) vs retention time (flow rate 0.5 mL/min) (■ = mol % PMMA and ▲ = mol % PI) for critical conditions of (a) PMMA and (b) PI; lines are representing the NMR projections (NMR chromatograms): solid lines = syndiotactic (rr) and atactic (mr) α-CH_3 of PMMA, dashed lines = olefinic 1,2-, 1,4-, and 3,4-PI.

time. In order to calculate the overall chemical composition of the block copolymer, it is necessary to determine the tacticity of PMMA and the content of the different PI microstructures. It is indicated in Figure 5 that the atactic triad (mr) of the α-CH_3 group of PMMA is overlapped with the CH_3 group of 1,2-PI, and the isotactic triad (mm) is overlapped with a CH_2 group of PI. (The content of the mm triad is very low and was neglected further on.) Overlappings are also found for the olefinic 1,2- and 1,4-PI protons. This was confirmed by off-line 2D-NMR. However, these specific proton intensities could be determined and used for calculating the CCD of the block copolymer. Unique signals of the polymer could be observed for the syndiotactic triads (rr) of PMMA and the olefinic 1,2- and 3,4-PI proton signals. The atactic triads (mr) and the content of 1,4-PI could be determined by appropriate subtractions. On the basis of this microstructure information the chemical composition distribution for both monomer units could be determined.

Figures 6 and 8 show the individual normalized elution curves (NMR chromatograms) for the PMMA triads and the PI isomers of samples 5 (predominantly 3,4-PI-*b*-PMMA) and 2 (predominantly 1,4-PI-*b*-PMMA). They also show the chemical composition as a function of retention time for the block copolymers at both critical conditions determined from the on-flow experiments.

Figure 6a provides monomodal distributions for all monomer units at critical conditions of PMMA for sample 5. In this case no differentiations between different species are possible. All curves show almost the same maximum. However, this separation clearly indicates that the sample does not contain PMMA homopolymer. Ethyl acetate suppression partially affects the mr triad intensity which is slighty diminished and shifted. At critical conditions of PMMA, a separation from PI homopolymer is not achieved and can cause strong deviations from the correct molar mass and chemical composition of the copolymer. The chemical composition was determined to be 54.3:45.7 mol % (PI/PMMA). This result corresponds to the bulk measurement (55.6/44.4 mol %) and indicates a higher PI content. Therefore, these conditions will provide wrong molar masses for the copolymer.

Furthermore, at critical conditions of PMMA, PI homopolymer cannot be detected since the molar masses of the homopolymer and the PI block are similar and, accordingly, they coelute. Consequently, a monomodal distribution of the PI moieties is observed in Figure 6a.

Using critical conditions of PI, monomodal distributions for the syndiotactic (rr) and atactic (mr) α-CH_3 groups of

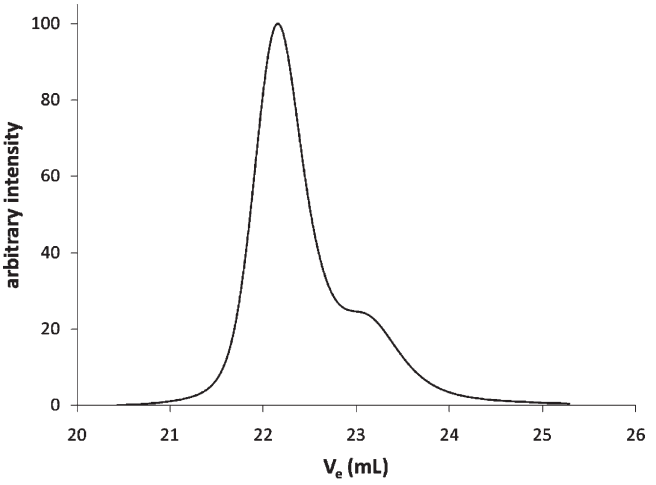


Figure 7. SEC chromatogram of sample 5 (RI detection, PSS SDV columns (500, 10^4, and 10^6 Å) 2 mg/mL, 100 μL injection volume, flow rate 1 mL/min).

PMMA are observed in Figure 6b. On the other hand, bimodal distributions for the olefinic 1,2-, 1,4-, and 3,4-PI components are found. This is a clear hint for the existence of a block copolymer fraction (retention time 9−11 min within the gray area) and PI homopolymer fraction (retention time 11−13 min). These NMR chromatograms provide 53.5 mol % block copolymer and 46.5 mol % PI homopolymer related to the total olefinic content (see also Table 3). On the basis of Figure 6b the correct molar mass of the PMMA block can be determined. The retention time of the first peak maximum of the rr and mr triads of the α-CH_3 groups of PMMA provides directly the molar mass of the PMMA block via the calibration curve of Figure 2. The molar mass of the PMMA block is 27.1 kg/mol. The first eluting part (9−11 min) provides also the true CCD of the separated block copolymer. The true average chemical composition of the copolymer is 38.7:61.3 mol % PI/PMMA. This PI content is significantly lower than the bulk measurements due to the separated PI homopolymer. Finally, the true molar mass of PMMA and the true CCD allows the calculation of the molar mass of the PI block.

The PI block length is determined to be 11.6 kg/mol. Consequently, the true molar mass of the copolymer can be calculated to be 38.7 kg/mol (see Table 3).

However, the existence of PI homopolymer is already indicated by the SEC analysis which shows a bimodal

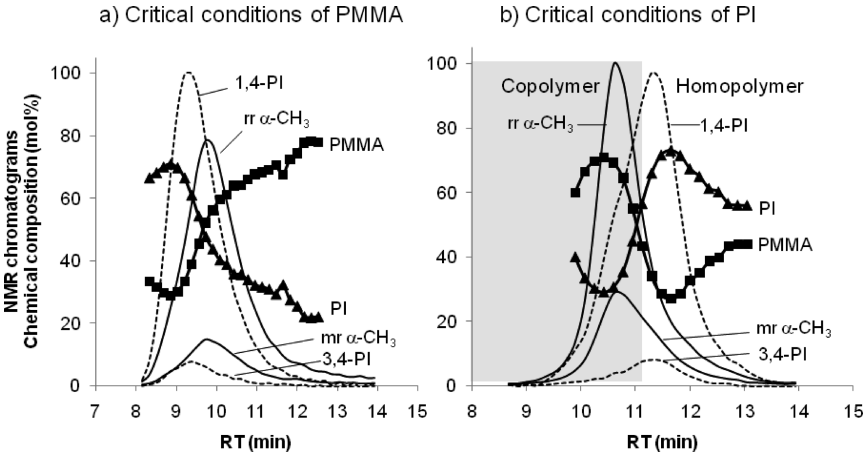


Figure 8. Chemical composition of the 1,4-PI-*b*-PMMA copolymer (sample 2) versus retention time (■ = mol % PMMA and ▲= mol % PI) for critical conditions of (a) PMMA and (b) PI; lines are the NMR projections (NMR chromatograms): solid lines = syndiotactic (rr) and atactic (mr) α-CH_3 of PMMA, dashed lines = olefinic 1,4- and 3,4-PI.

Table 3. Molar Masses and Average Chemical Compositions of PI-b-PMMA Determined by ^{1}H NMR and SEC and LCCC-NMR at Critical Conditions of PI[a]

sample	M_p of PMMA (kg/mol) by SEC and ^{1}H NMR	M_p of PI (kg/mol) by SEC and ^{1}H NMR	isoprene/MMA (mol %) by ^{1}H NMR	M_p of PMMA (kg/mol) by LCCC-NMR	M_p of PI (kg/mol) by LCCC-NMR	M_p of PI-*b*-PMMA (kg/mol) by LCCC-NMR	isoprene/MMA (mol %) by LCCC-NMR	content of PI homopolymer (mol %)
1	2.3	16.6	91.4/8.6	**1.5**	**8.7**	**10.2**	**89.7/10.3**	**14.9**
2	11.4	8.0	50.7/49.3	**10.9**	**5.4**	**16.3**	**41.9/58.1**	**3.9**
3	78.8	10.1	15.9/84.1	**83.9**	**7.3**	**91.2**	**11.3/88.7**	**9.2**
4	87.1	20.2	25.4/74.6	**101.0**	**6.5**	**107.5**	**8.7/91.3**	**52.4**
5	32.8	27.9	55.6/44.4	**27.1**	**11.6**	**38.7**	**38.7/61.3**	**46.5**

[a] Bold characters represent the LCCC-NMR. Content of PI homopolymer is referred to the total PI.

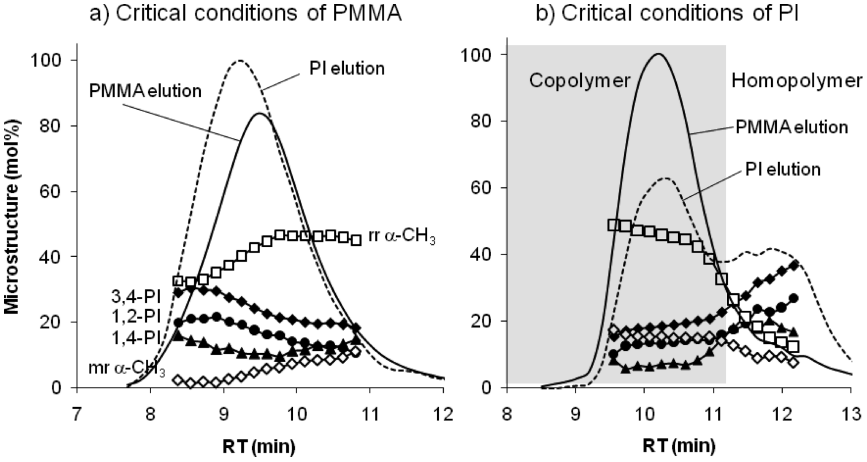


Figure 9. Microstructure distribution referred to the entire 3,4-PI-*b*-PMMA (sample 5) at critical conditions of (a) PMMA and (b) PI; □ = mol % of syndiotactic (rr) α-CH_3 of PMMA, ◇ = mol % of atactic (mr) α-CH_3 of PMMA, ◆ = mol % of olefinic 3,4-PI, ● = mol % of olefinic 1,2-PI, ▲ = mol % of olefinic 1,4-PI, solid line = NMR chromatogram of total PMMA, dashed line = NMR chromatogram of total PI.

distribution for sample 5 (see Figure 7). (A detailed SEC-NMR analysis will be presented in a forthcoming paper.)

Figure 8 shows the NMR chromatograms of the individual microstructures as well as the chemical compostion of sample 2 for both critical conditions. Sample 2 contains a PI block that is predominantly built of 1,4-PI units. In this case, critical conditions of PMMA provide again monomodal distributions for all microstructures (see Figure 8a). However, the sample is much more heterogeneous indicated by the shifted elution curves of PI and PMMA. PI elutes first (at the region of higher molar masses), whereas PMMA elutes later (region of smaller molar masses) due to the critical conditions of PMMA. As for sample 5, no PMMA homopolymer is visible.

Figure 8b shows different behavior compared to Figure 8a. It also illustrates a strong heterogeneity (PMMA block elutes first and PI later). The main difference is the shoulder of the 1,4- and 3,4-PI elution at 10.6 min (M_p of the PI block). This region (about 9−11 min) indicates the elution of the block copolymer. The area above 11 min presents PI homopolymer elution and is partially overlapped with the region of the copolymer.

Moreover, Figures 9 and 10 show the distributions of the PMMA triads and the PI microstructures which can be directly calculated from the individual NMR chromatograms. They can be calculated according to the total copolymer (Figure 9) or separately for the individual blocks

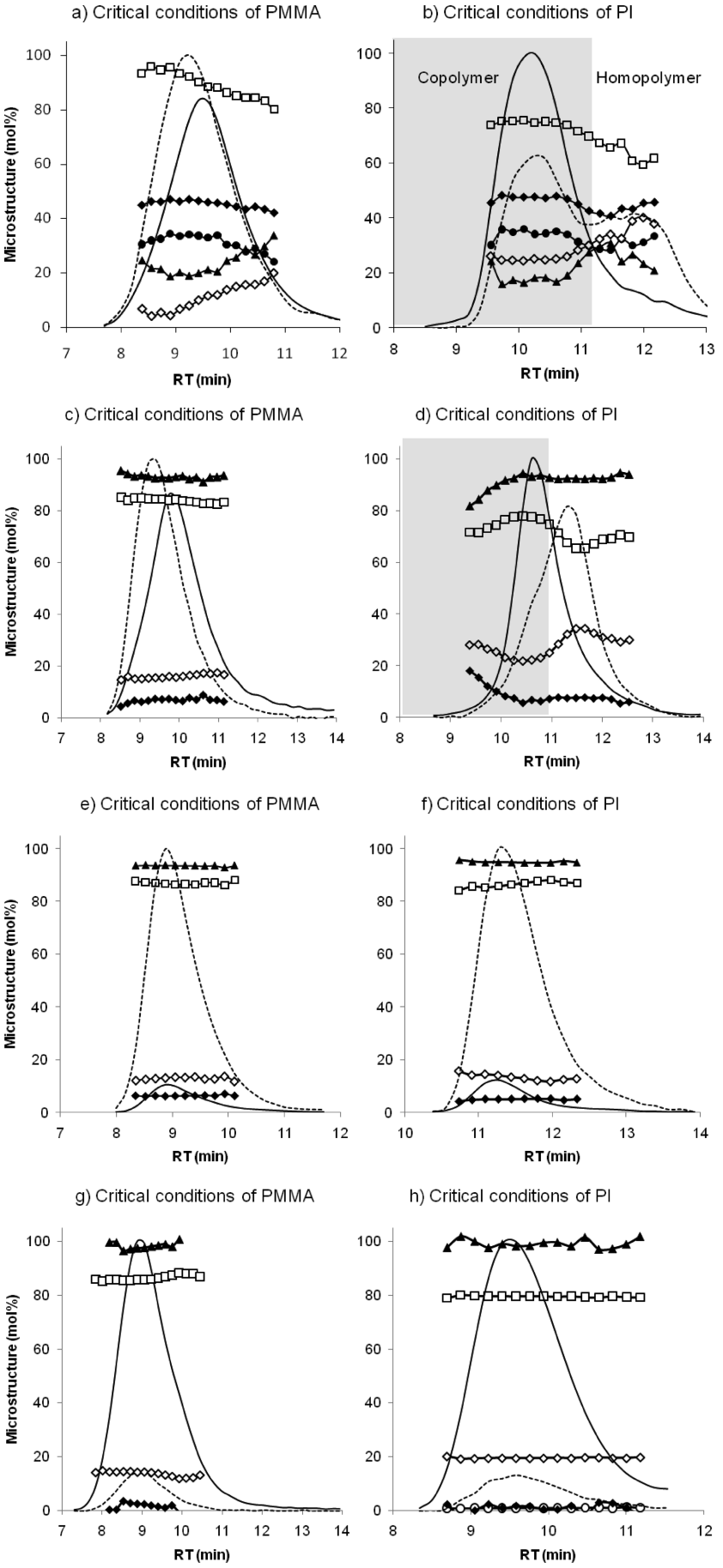


Figure 10. Continued

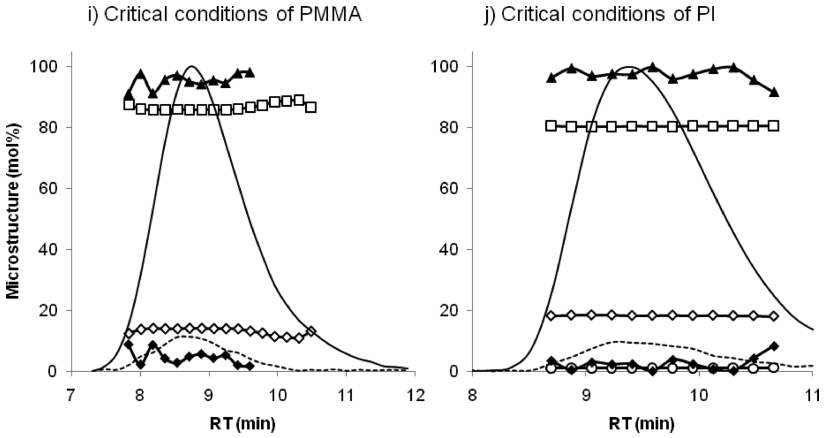


Figure 10. Microstructure distribution referred to the individual blocks of 1,4-PI-*b*-PMMA and 3,4-PI-*b*-PMMA at critical conditions of PMMA and PI: sample 5 (a, b), sample 2 (c, d), sample 1 (e, f), sample 3 (g, h), sample 4 (i, j) (□ = mol % of syndiotactic (rr) α-CH_3 of PMMA, ◇ = mol % of atactic (mr) α-CH_3 of PMMA, ● = mol % of olefinic 1,2-PI, ▲ = mol % of olefinic 1,4-PI, ◆ = mol % of olefinic 3,4-PI, solid line = NMR chromatogram of total PMMA, dashed line = NMR chromatogram of total PI.

(Figure 10). The calculation referred to the total chemical composition is only demonstrated for sample 5 (Figure 9a,b). Figure 10 contains the microstructure distribution related to the individual blocks for all samples and both critical conditions. These figures illustrate more clearly the behavior of the microstructure for both blocks.

The copolymer region between 9 and 11 min in Figure 9a indicates a slight increase of the rr and mr triads with increasing retention times which finally results in the increase of the total PMMA content with decreasing molar masses. The opposite tendency is seen for the polyisoprene block. 1,2-, 1,4-, and 3,4-PI are decreasing with decreasing molar masses. Figure 9b shows the opposite tendency for PI and PMMA.

This behavior is caused by the critical conditions and is an indication for a chemical heterogeneity of the copolymer.

Figure 10 shows the dependence of the microstructures on the retention times (or molar masses) as well as the entire PMMA and PI elution curves derived from the individual NMR chromatograms. These figures provide very important information:

First, the existence of block copolymer and homopolymer can be established. Using critical conditions of PI, the differentiation of block copolymer and PI homopolymer would be possible. Table 3 shows the estimated contents of homopolymer PI for all copolymer samples. The content of PI homopolymer is compared to the total content of PI. Using critical conditions of PMMA, no hints for the existence of PMMA homopolymer are given.

Second, the microstructure distribution of both the copolymer and the existing homopolymer can be directly measured for each PMMA triad and PI isomer. The total elution curves for PMMA and PI can be constructed, which reflect the chemical composition distribution.

Third, the molar masses of the PI and the PMMA blocks can be directly determined. The true molar mass of the PMMA block can be determined at critical conditions of PI. Furthermore, critical conditions of PI also provide the true chemical composition of the copolymer which finally can be used for the calculation of the true molar mass of the PI block.

Table 3 shows the comparison of the molar masses and average chemical compositions determined by LCCC-NMR at critical conditions of PI as well as off-line ^{1}H NMR and SEC. It can be seen from this table that the molar masses of the PMMA blocks are comparable for both methods. The molar mass of the PMMA block is directly determined from the maximum of the PMMA elution. Since no PMMA homopolymer was observed, comparable data were obtained for the PMMA blocks from LCCC-NMR as well as ^{1}H NMR and SEC. In order to determine the true molar mass of the PI block, the average chemical composition was measured from the LCCC-NMR on-flow runs via the elution traces of the copolymer region only. In this case, the PI homopolymer is separated and the true chemical composition of the block copolymer is obtained. Table 3 shows also the chemical compositions. It is clearly seen that LCCC-NMR provides lower contents of PI than bulk measurements. LCCC-NMR provides the correct chemical composition because no PI homopolymer is affecting the CCD. As the consequence, the true molar mass of the PI block can be calculated from the chemical composition and the molar mass of PMMA. Table 3 shows that the molar mass of the PI block is smaller than calculated by SEC and ^{1}H NMR. The data for critical conditions of PMMA are not presented due to the fact that PI standards of higher molar masses (> 30 kg/mol) are insoluble in ethyl acetate. In addition, coelution of PI homopolymer and block copolymer takes place.

As already discussed, Figure 10a,b also confirms the absence of PMMA homopolymer (total PMMA elution in Figure 10a) and the presence of PI homopolymer (total PI elution in Figure 10b). It also demonstrates the distribution of the microstructure of sample 5. Whereas the syndiotactic triads decrease (see Figure 10a), the atactic triads are increasing with increasing retention times. The isomeric PI moieties are slightly changing during the elution but appear almost equal at the beginning and the end of the polymer elution. Figure 10b demonstrates the true microstructure behavior of each copolymer part. It is evident from this figure that the microstructure is almost evenly distributed within the two blocks. Therefore, the change of the microstructure of the PI block in Figure 10a is affected by the microstructure of the PI homopolymer. Because of the fact that no PMMA homopolymer exists, the changes of the PMMA microstructure reflect a heterogeneous microstructure distribution within the PMMA block. In case of critical conditions of PMMA, the PMMA block has the highest syndiotactic tacticity at the highest molar masses, which decreases gradually with decreasing molar masses. Figure 10b is also used for the correct determination of the molar mass of the PMMA block. The maximum of the PMMA elution provides directly the molar mass by using

Table 4. Microstructures of PI-b-PMMA for Off-Line ^{1}H NMR and LCCC-NMR

sample	1,2/1,4/3,4-PI (mol %) by ^{1}H NMR	1,2/1,4/3,4-PI (mol %) by LCCC-NMR	mm/mr/rr-PMMA[a] (mol %) by ^{1}H NMR	mm/mr/rr-PMMA[a] (mol %) by LCCC-NMR
1	0/94.4/5.6	0/94.9/5.1	0/17.7/82.3	0/13.6/86.4
2	0/93.4/6.6	0/92.8/7.2	0/22.8/77.2	0/24.3/75.7
3	0/94.4/5.5	0/98.9/1.1	1.0/17.5/81.8	1.0/19.5/79.5
4	0/94.6/5.4	0/97.6/2.4	0.2/16.4/83.5	1.1/18.4/80.5
5	34.4/19.3/46.3	34.2/18.5/47.3	0/19.4/80.6	0/25.3/74.7

[a] mm = isotactic, mr = heterotactic, rr = syndiotactic triads.

the calibration of Figure 2. The true chemical composition of the block copolymer can be calculated from the total elution curves for each retention time within the gray area (9−11 min). With the aid of the molar mass of PMMA and the chemical composition, the correct molar mass of the PI block can be determined. Table 3 shows the molar masses of all block copolymers.

Figure 10d also indicates coelution of the block copolymer and PI homopolymer for sample 2. The shoulder of the PI elution curve corresponds to the maximum of the PI block. This figure shows also a strong dependence of the microstructure. Both the contents of syndiotactic triads and 1,4-PI increase with increasing retention times until the shoulder. The changes afterward cannot easily be evaluated due to the coelution with the homopolymer.

Figure 10e gives also no indication for the presence of PMMA homopolymer. There might be a small indication for PI homopolymer. The microstructure is constant during the whole elution for critical conditions of PMMA. A slight increase of the syndiotacticity is observed for critical conditions of PI.

Samples 3 (Figure 10g,h) and 4 (Figure 10i,j) are very similar. Both samples show a very homogeneous distribution. In this case, no homopolymers can be observed and no change of the microstructure is found. These polymers consist of about 90% PMMA. Therefore, the isotactic component is even visible.

With respect to the critical conditions, it should also be noted that the high injection concentrations will also broaden the elution peaks and can cause a tailing to higher elution volumes as well as decrease the sample recovery from the column for higher molar masses. This problem was studied by Teraoka regarding osmotic effects and viscous fingering phenomena.[38] Therefore, it might be possible that small amounts of PMMA homopolymer could be present but were not visible with LCCC-NMR at critical conditions of PMMA. In the case of critical conditions of PI we expect a high recovery due to the fact that high molar masses of PI and PMMA could be used for the calibrations. Berek has shown an alternative for LCCC to separate even small amounts of a B homopolymer from an AB block copolymer[39,40] by using LC under limiting conditions.

The average microstructures of both the bulk measurements and LCCC-NMR are presented in Table 4.

All samples have a highly syndiotactic PMMA block. The isotactic parts could only be resolved for samples 3 and 4. It is found from Table 4 that the tacticity is comparable for the bulk and the LCCC-NMR measurements.

Conclusion

It was shown that LCCC-NMR is a powerful tool for the analysis of block copolymers. In particular, it allows the separation of the parent homopolymers and the block copolymers. Because of the fact that NMR is an accurate concentration detector for both monomer units, it provides the correct chemical composition of the copolymer. Using the true chemical composition, it is finally possible to determine the true molar masses of both blocks. In case of PI-*b*-PMMA copolymers it was possible to develop critical conditions by using only single solvents as mobile phases and varying the column temperature. A full microstructure determination during the chromatographic elution is the basis for the analysis of the block copolymer. The chemical composition and the molar masses of the PMMA and PI blocks could be determined via the individual microstructure distributions.

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