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HPLC-^{1}H-NMR Characterization of Polystyrene-*block*-Polyisoprene Copolymers: LCCC-^{1}H-NMR Using a Single Mobile Phase

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Summary: The analysis of PS-*b*-PI copolymers, synthesized by sequential living anionic polymerization was conducted by on-line hyphenation of liquid chromatography at critical conditions (LCCC) and proton nuclear magnetic resonance spectroscopy (^{1}H-NMR). Critical conditions were established for polyisoprene using 1,4-dioxane as the mobile phase and varying the column oven temperature. At these critical conditions, the polyisoprene homopolymer formed during synthesis was separated from the copolymer. The molar mass of the PS block, the chemical composition of the block copolymers as well as the microstructure of PI were determined in a single experiment.

Keywords: HPLC-NMR coupling; liquid chromatography at critical conditions; microstructure analysis; PS-*b*-PI copolymers; sequential living anionic polymerization

Introduction

Block copolymers comprise two or more different homopolymer subunits linked by covalent bonds. They are complex macromolecular systems characterized by distributions in chemical composition, molar mass and chain architecture. Due to these distributions they are interesting materials for research in the field of fundamental and applied polymer science. By using suitable and well defined polymerization procedures the morphology and the physical properties of block copolymers can be tailored. Recent research in block copolymers suggests that they may be useful in creating self-constructing fabrics with potential utility in semiconductor arrays (for example, computer memory devices) by assembling fine details atop a structured base created using conventional microlithography methods.[1] Linear block copolymers are typically prepared by living anionic polymerization where sequential monomer addition is used.[2]

Many research groups have shown that liquid chromatography at the critical point of adsorption is a powerful tool for separating block copolymers.[3–7] This technique is also called liquid chromatography at critical conditions where one block of the copolymer is considered to be chromatographically invisible whereas the other block elutes either under size exclusion chromatography (SEC) or liquid adsorption chromatography (LAC) conditions. By using this technique it is possible to determine the block lengths of the individual blocks in the copolymer.[8] There are some applications of liquid chromatography at critical conditions for the analysis of block copolymers. Block copolymers of poly(decyl methacrylate-*b*-methyl methacrylate),[9] poly(methyl methacrylate-*b*-tert-butyl methacrylate)[10] and poly(dimethylsiloxane-*b*-styrene) were analyzed by using solvent mixtures.[11]

A number of articles were published by Chang et al. on the analysis of PS-*b*-PI

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copolymers. Different techniques such as liquid chromatography at critical conditions, gradient high performance liquid chromatography (HPLC) and temperature gradient interaction chromatography (TGIC) were used for the separation of these block copolymers with regard to chemical composition.[12–16] Chang et al. also used a single solvent as mobile phase to establish the critical conditions and to analyze PS-*b*-PI copolymers.[17] Macko et al. also used a single solvent to establish the critical conditions for a number of homopolymers.[18]

The on-line coupling of HPLC and NMR forms a robust tool for the investigation of complex mixtures. It has been shown that the combination of chromatographic separation and nuclear magnetic resonance spectroscopy can directly provide information on the individual components of the mixture. This technique is well established for the analysis of mixtures in the pharmaceutical industry. The main application is the analysis of metabolites and natural products. Few research groups have worked and published results on HPLC-NMR coupling for the analysis of complex polymer mixtures. Krämer et al. analyzed random copolymers of poly[styrene-co-(ethyl acrylate)] by gradient HPLC-NMR.[19] LCCC-NMR was also used for the analysis of poly(ethyl methacrylate)s regarding tacticity.[20] The functionality type distribution of poly(ethylene oxide)s was studied by LCCC-NMR.[21–22] The present authors first published the application of on-flow LCCC-NMR for the characterization of poly(styrene-*b*-methyl methacrylate) copolymers and blends of homopolymers at critical conditions of both PS as well as PMMA using solvent mixtures.[23–24] An application of LCCC-NMR for the characterization of PI-*b*-PMMA copolymers using a single solvent was also published by the present authors.[25] The same authors also used LCCC-NMR for the characterization of blends of PS and PI homopolymers as well as PS-*b*-PI copolymers. The critical conditions were established using solvent mixtures.[26–27] By using LCCC-NMR, blends of PI having different microstructures were separated and analyzed by the authors.[28] Most recently comprehensive two-dimensional liquid chromatography was coupled on-line with ^{1}H-NMR for the analysis of poly(ethylene oxide)s having different endgroups and chain length distributions.[29]

In the present study PS-*b*-PI copolymers will be analyzed by the on-line hyphenation of liquid chromatography at critical conditions with ^{1}H-NMR. The block copolymers were synthesized by sequential living anionic polymerization. Critical conditions were established for the PI block of the copolymer using a single solvent and varying the column oven temperature. The chemical composition distribution of the block copolymers and the molar mass of the PS block will be determined. The microstructure of PI will be identified and quantified accordingly.

Experimental Part

Critical Conditions of PI

For the critical chromatography experiments an Agilent 1100 HPLC system (Böblingen, Germany) equipped with a quaternary pump (G1311A), vacuum degasser (G1322A), column oven (G1316A), an autosampler (G1313A), variable wavelength UV detector (G1314A) and ELS (Evaporative light scattering) detector PL 1000 (Polymer Laboratories/Varian) was utilized. The critical conditions of PI were established by using a set of non-polar stationary phase columns from Macherey-Nagel (Düren, Germany) Nucleosil C_{18} 300 Å-5 μm and C_{18} 1000 Å-7 μm with column sizes of 250 × 4 mm i.d. The concentration of the PI samples was 0.5 mg· mL^{-1} and the volume injected was 10 μL. HPLC grade 1,4-dioxane was used as the mobile phase at a flow rate of 0.5 mL· min^{-1}. By varying the temperature of the column oven the critical conditions were established.

On-line Hyphenation of LCCC and ^{1}H-NMR

The outlet of the UV detector of the Agilent HPLC unit was connected to the peak sampling unit (BPSU-12) from Bruker Biospin GmbH, Rheinstetten, Germany. The peak sampling unit was then connected with capillaries to the continuous flow probe in the cryomagnet containing a 60 μL flow cell. The probe was a ^{1}H {^{13}C} inverse detection probe equipped with a shielded pulsed field-gradient coil. The gradient strength was 53 G· cm^{-1}. The 90° ^{1}H pulse was 4.7 μs. The pulse technique WET (Water suppression enhanced through *T1* effects)[30] was applied to HPLC grade 1,4-dioxane used for chromatography. One frequency was suppressed. On-flow experiments were performed for studying the critical conditions. 8 scans per free induction decay (FID) were acquired with an acquisition time of 1.1 s (16 kb data points) and a relaxation delay of 0.1 s. In case of the homopolymers the concentration was 10 mg· mL^{-1} and for the block copolymers 20 mg· mL^{-1}. The volume injected was 50 μL and the flow rate was maintained at 0.5 mL· min^{-1}.

Results and Discussion

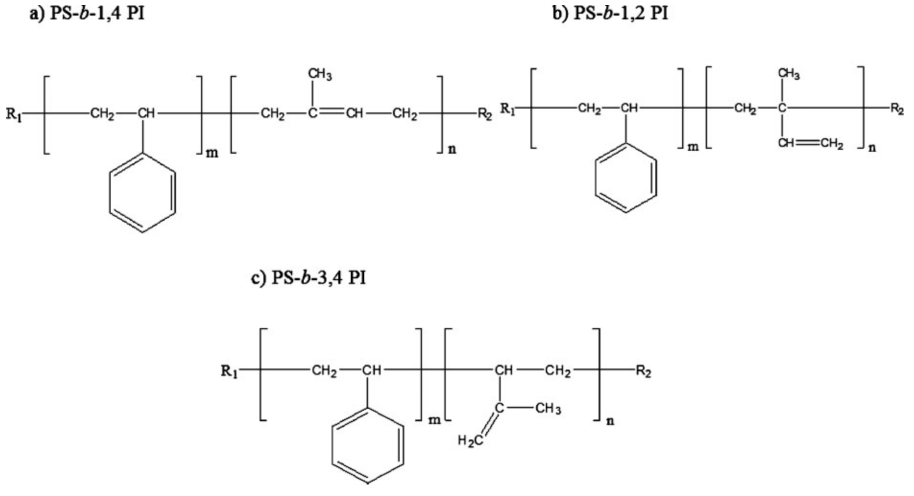
Table 1.
Total molar mass of PS-*b*-PI copolymers estimated by SEC and the average chemical composition determined by ^{1}H-NMR, sma: sequential living anionic polymerization, molar masses are polystyrene equivalents.

Sample	Name	M_p by SEC (kg· mol^{-1})	Styrene/ isoprene by ^{1}H-NMR (mol %)
1	(38–44) sma	118.5	38.6/61.4
2	(54.3–25) sma	101.9	56.1/43.9
3	(18.5–57.8) sma	107.3	16.2/83.8

The block copolymers given in Table 1, synthesized by sequential living anionic polymerization[2] were used for the analysis. The total molar mass of the block copolymers was estimated by SEC using THF as solvent and the average chemical composition was determined by ^{1}H-NMR using deuterated THF as solvent.

Polyisoprene shows different microstructures depending on the conditions used for the synthesis by sequential living anionic polymerization. Scheme 1 shows PS-*b*-PI copolymers having different structures of the PI unit.

During the synthesis of block copolymers by sequential living anionic polymerization there is a possibility for the formation of byproducts as well as homopolymers. In order to separate these impurities from the block copolymers chromatographic separation is necessary. Chromatography at critical



Scheme 1.
Structure of PS-*b*-PI copolymers with (a) 1,4-PI, (b) 1,2-PI and (c) 3,4-PI units.

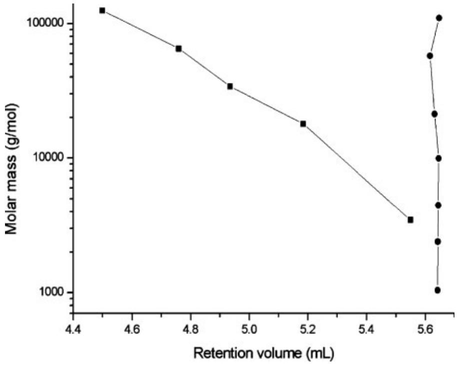


Figure 1.
Critical diagram for PI. Stationary phase: Nucleosil C_{18} 300 Å-5 μm and C_{18} 1000 Å-7 μm; Mobile phase: 1,4-dioxane; column temperature: (●) T = 54 °C (LCCC for PI) and (■) T = 54 °C (SEC for PS).

conditions will be used to separate the impurities formed during synthesis from the block copolymers. In the present case chromatography at critical conditions is established for the PI block of the copolymer.

Figure 1 shows the critical diagram for PI depicting molar mass versus retention volume. The critical conditions of PI are obtained by using a set of non-polar stationary phase Nucleosil C_{18} 300 Å-5 μm and C_{18} 1000 Å-7 μm columns. The mobile phase was 1,4-dioxane and the flow rate was maintained at 0.5 mL· min^{-1} and the column oven temperature was varied. At a temperature of 54 °C the critical conditions of PI were found were all the PI standards (M_w: 2.6, 4.8, 8.4, 9.8, 20, 32.2, 45.9 and 94.4 kg· mol^{-1}) elute at the same elution volume irrespective of their molar mass. PS standards having different molar masses ($M_{w:}$ 3.5, 17.9, 34, 65, 125 kg· mol^{-1}) were measured at this temperature. The PS standards show SEC behavior where the lower molar mass standards are retained longer in the stationary phase than the higher molar mass standards. These PS standards were then used to construct a molar mass calibration curve. This curve was used to calculate the molar mass of the PS block of the copolymer.

The proton NMR spectra of the copolymer measured in deuterated tetrahydrofuran (a) and protonated 1,4-dioxane (b) are shown in Figure 2. 1,4-dioxane is a good

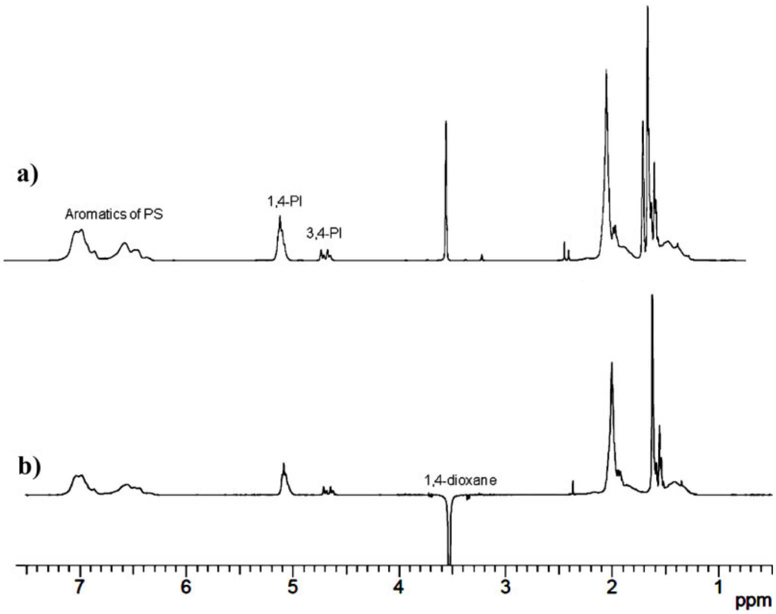


Figure 2.
^{1}H-NMR spectra of PS-*b*-PI (sample 1): (a) deuterated THF and (b) protonated 1,4-dioxane (the solvent signal of 1,4-dioxane was suppressed by WET).[30]

solvent for HPLC-NMR coupling since it can be used for HPLC separation of the copolymer from the homopolymer formed during synthesis. The solvent signals do not overlap with the signals of interest of the copolymer which can be used to calculate the chemical composition as well as the microstructure of the copolymer. Since protonated HPLC grade 1,4-dioxane was used, the signal intensity of the solvent is a thousand times larger than the signals of interest of the copolymer. In order to see the signals of the copolymer the signal of 1,4-dioxane is suppressed by using the NMR solvent suppression technique WET.[30]

The LCCC-NMR on-flow plot of sample 1 measured at the critical conditions of PI is shown in Figure 3. At the critical conditions of PI the PI homopolymer formed during synthesis is separated from the copolymer. In the on-flow plot the X-axis represents the proton chemical shift in ppm whereas the Y-axis represents the retention time in minutes. The proton chemical shift region from 1.0 to 7.5 ppm is displayed. As expected there is an absence of PI homopolymer since the block copolymers are synthesized by sequential living anionic polymerization where the first synthesized block is PS followed by the second PI block. The block copolymer shows co-existing signals in the aliphatic, olefinic and aromatic region in the on-flow plot. The on-flow plot also shows a trace of the 1,4-dioxane signal at 3.58 ppm after solvent suppression. The aromatic signals of PS (6.3–7.1 ppm) and the olefinic proton signals of 1,4-PI (5.1 ppm) and 3,4-PI (4.65–4.75 ppm) are used to calculate the chemical composition of PS-*b*-PI copolymer. Sample 1 shows the presence of the copolymer and the absence of PI homopolymer.

Table 2 shows a comparison of the molar masses of the PS blocks of the copolymers estimated by SEC and off-line NMR as well as LCCC-NMR coupling. The values obtained are quite close to each other. The chemical composition of the copolymers is also calculated from the LCCC-NMR on-flow plots by adding the proton NMR traces of the aromatic protons of PS

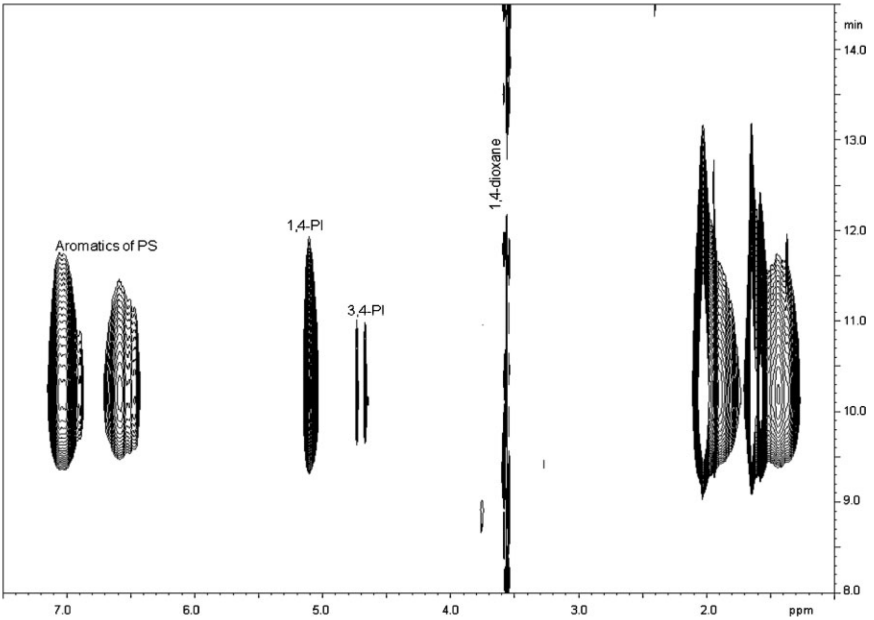


Figure 3.
LCCC-NMR on-flow plot of sample 1 measured at the critical conditions of PI (concentration of copolymer 20 mg · mL^{-1}, injection volume 50 μL, flow rate 0.5 mL · min^{-1}).

Table 2.

Molar mass of the PS block calculated by SEC and off-line NMR, molar mass of the PS block by LCCC-NMR, chemical composition of the block copolymer and the total calculated molar mass of the copolymer at the critical conditions of PI.

Sample	M_p of PS block by SEC and NMR (kg· mol^{-1})	M_p of PS block by LCCC-NMR (kg· mol^{-1})	Styrene/isoprene of copolymer by LCCC-NMR (mol %)	M_P of block copolymer calculated (kg· mol^{-1})
1	58.1	40	35.9/64.1	86.7
2	67.5	62.9	64.2/35.8	85.8
3	24.5	12.0	11.8/88.2	70.6

and the olefinic protons of PI. The total molar masses of the copolymers are calculated by using the molar mass of the PS block and the chemical composition obtained by LCCC-NMR.

The chemical composition of the copolymers versus retention time measured at the critical conditions of PI is depicted in Figure 4. From the LCCC-NMR on-flow run the microstructure of the PI blocks in the samples are determined. The PI block of the samples is not pure but contains a mixture of large amounts of 1,4-PI units, small amounts of 3,4-PI units and does not show 1,2-PI units. All the samples predominantly show the presence of 1,4-PI units. The microstructure of PI remains constant with retention time. NMR chromatograms are constructed from the LCCC-NMR on-flow runs by taking the projections of the aromatic region of PS (dashed line) and the olefinic regions of 1,4-PI (solid line) and 3,4-

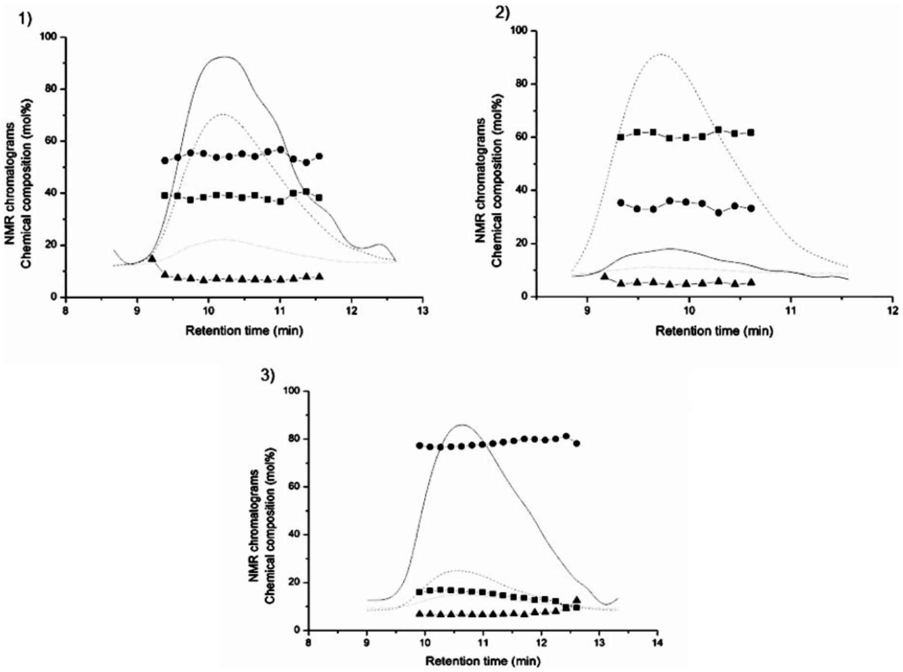


Figure 4.

Chemical composition of PS-*b*-PI copolymers (samples 1–3) versus retention time (■ = mol % PS, ● = mol % 1,4-PI and ▲ = mol % 3,4-PI) for critical conditions of PI; lines are the NMR projections (NMR chromatograms): dashed line = aromatic region of PS, solid line = olefinic region of 1,4-PI and dotted line = olefinic region of 3,4-PI.

PI (dotted line). The chromatograms of all the samples show a monomodal distribution indicating that the samples do not contain PI homopolymer. The chemical compositions of the copolymers are calculated from the aromatic protons of PS (■ = mol % PS) and the olefinic protons of PI (● = mol % 1,4-PI and ▲ = mol % 3,4-PI). All the copolymers are homogenously distributed with regard to the chemical composition. The retention time of the PS projection profiles (aromatic protons) can be used for calculating M_p of the PS block.

Conclusion

It can be finally concluded that the on-line hyphenation of HPLC with ^{1}H-NMR is a robust tool for the analysis of complex polymer mixtures. The separation of byproducts formed during synthesis from the main products as well as the identification of the microstructure can be carried out in a single experiment. The on-line coupling of HPLC and ^{1}H-NMR is complicated, therefore, the use of a single mobile phase is advantageous since only one solvent signal has to be suppressed. The molar mass of the PS block, chemical composition of the block copolymers, and microstructure of the PI block can be calculated by using on-line LCCC-NMR coupling.

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