Interactive liquid chromatography for the characterisation of polymers
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CHAPTER SIX

CHARACTERISATION OF BLOCK AND RANDOM POLYSTYRENE-POLYMET HYL METHACRYLATE COPOLYMERS USING ON-LINE GRADIENT LC-NMR.

Abstract

Block and random copolymers of polystyrene and polymethyl methacrylate were characterised by reversed-phase gradient liquid chromatography coupled on-line to a nuclear-magnetic-resonance spectrometer. The polymers were separated according to chemical composition and the relative concentration of the two monomers in the copolymer was monitored using the appropriate NMR signals. For random copolymers, a broad low peak was obtained for the methyl ester protons of the MMA monomer, making accurate quantitation of this signal difficult. The same signal in equivalent block copolymeric samples was much sharper, allowing a better determination of the chemical composition. For both the random and the block copolymers, the aromatic protons of the styrene monomers were quantifiable, as long as the signal-to-noise ratio was high enough.

Introduction

Synthetic polymers consist of distributions of molecules that vary in size and in the case of more complex polymers, chemical composition and molecular architecture. These distributions are all directly influenced by experimental parameters in the synthetic process (such as temperature, monomeric concentrations, etc.) and can significantly affect the physical and chemical properties of the final polymer product. It is therefore necessary to monitor these distributions in order to optimise synthetic processing parameters and ultimately to obtain a polymer product with the required chemical and physical properties.

Liquid chromatography of polydisperse macromolecules.

Conventional characterisation of polymers usually involves size-exclusion chromatography (SEC), a chromatographic technique that separates on the basis of the size of the macromolecules in a given (strong) solvent. While SEC is a valuable tool for the determination of size-based distributions (e.g. molar-mass distributions), it cannot identify chemical differences between molecules and it is not suitable for the characterisation of distributions other than size. In this case, separation techniques
such as gradient and isocratic liquid chromatography i.e. interactive LC (iLC) are better options, because these separate on the basis of molecular interactions between the polymer and the mobile and stationary phases and can be used to characterise chemical differences (1, 2, 3, 4).

By tailoring the chromatographic system (i.e. stationary and mobile-phases) to the particular requirements of an analysis, iLC can be used to characterise distributions of chemical composition, functional groups, tacticity, molar mass, etc. Isocratic iLC is useful for oligomeric and functional-group separations of low-molar-mass polymers (5). It is also used for molar-mass-independent separations of macromolecules at the so-called ‘critical point’, the mobile-phase composition where the free-energy effect (ΔG) of a given monomer is zero. Under these specific conditions, the monomeric unit will no longer influence retention. The technique can be used to characterise block-length, end-group and tacticity distributions (6, 7, 8). However, experimentally, it remains challenging (the critical point is very sensitive to minor changes in solvent quality, temperature and stationary-phases) (9, 10) and to date the applications of critical chromatography remain relatively scant.

Gradient chromatography is a much more robust and versatile technique, especially for the characterisation of chemical differences in high-molar-mass polymers. ‘Pseudo-critical’ (gradient) conditions can be found where the effect of molar mass on retention becomes negligible, allowing separations that are independent of molar mass (11).

**Nuclear-Magnetic-Resonance Spectrometry**

NMR is one of the most powerful techniques available for the elucidating the structure of unknown compounds. Samples are either introduced directly in the probe, or fractionated prior to introduction to remove any impurities that may be present. Until recently, the direct coupling of NMR to liquid chromatography has been problematic, mainly due to the relative insensitivity of the technique and also to the high cost of the deuterated solvents that are required to avoid overwhelming signals from protons in the mobile-phase. However, recent improvements in NMR probe design (12, 13) and solvent-suppression techniques (14, 15) have opened the way for direct coupling of LC to NMR (16).

There are two options for on-line LC-NMR, i.e. stopped flow and continuous flow. In stopped-flow experiments, a peak (monitored for example by a UV detector) is eluted from the LC column into the NMR probe head. The pump of the LC is then stopped so that the eluted analyte remains in the NMR probe. The flow is stopped for as long as is necessary to obtain a reasonable signal-to-noise ratio. This technique is suitable for well-resolved, unimolecular analyte peaks. If the resolution
between peaks is not good enough, or if the chromatographic peak consists of a distribution of analyte molecules (such as for a polydisperse sample), stopped-flow experiments are not practical. In continuous-flow LC-NMR experiments, the LC pump works continuously. Analyte concentrations must be high enough and residence-times in the NMR probe long enough to achieve acceptable signal-to-noise ratios. Continuous-flow LC-NMR is more suitable for the characterisation of polydisperse samples, because, in this case, the chromatographic peaks consist of a distribution of analyte molecules, in terms of, for example, molar mass or chemical composition or a combination of a number of such distributions. For these types of samples, stopped-flow experiments are difficult, because the change in composition must be monitored across an eluting peak.

Applications of LC-NMR for polymer characterisation

While NMR as a stand-alone technique can only measure the averages of distributions present in a synthetic polymer, LC coupled to NMR allows the direct determination of the distributions, without the need for any standards (which are often difficult to obtain). Coupling with a size-based separation such as SEC can be used for the absolute determination of MMDs of uniform isotactic homopolymers (17, 18). The number-average degree of polymerisation is calculated from the intensity of the NMR resonance of an end-group relative to a repeating unit. Information regarding the tacticity of stereo-irregular polymers can be obtained by coupling either SEC or an iLC technique to NMR. Ute et al. coupled SEC to NMR for the separation of pure stereocomplexes from mixtures of uniform isotactic and syndiotactic PMMA (19). Kitayama et al. characterised the tacticity distribution of polyethyl methacrylates using critical chromatography coupled to NMR (20). LC-NMR can also be used to characterise the chemical composition of copolymers by measuring the change in the relative intensities of resonating groups from each of the monomeric units present in the copolymer. Kramer et al. investigated the chemical heterogeneity of high-conversion poly[styrene-co-ethyl acrylate] by online SEC-NMR. Their results indicated that the blocky character of the polymer increased with increasing molecular size (21).

By coupling with a chromatographic technique that separates according to chemical differences, the chemical-composition distribution (CCD) can be determined (22). Functionality-type distributions (FTD) can similarly be characterised by separating according to functionality and measuring the relative intensities of the functional group and the polymeric chain. Pasch et al. characterised a low-molar-mass polyethylene oxide according to both its functional end groups and its molar mass, using isocratic iLC coupled on-line to NMR (23).
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Coupling considerations

Continuous flow LC-NMR requires some optimisation for a successful coupling of the seemingly incompatible techniques. In terms of sensitivity, NMR probes need to be much more sensitive for continuous-flow work. Unlike in batch and stop-flow experiments, there is only a limited time available to scan the analyte molecules (limited by the flow-rate of the LC system and the cell volume of the NMR probe).

Sample amounts in the detector cell must also be significantly higher in NMR than in conventional UV or ELSD detection. This is achieved by increasing the amount of sample injected and the volume of the detector cell. Increased sample concentration or injection volume can lead to overloading of the solute on the stationary-phase. For high-molar-mass analytes, however, overloading is not commonly a problem, because the molecules tend to be either fully retained or fully unretained, depending on the strength of the mobile phase (i.e. there is very little partitioning of the molecules between the mobile and the stationary phases). Overloading effects are primarily seen as an increased 'breakthrough' peak, i.e. polymeric sample that is eluted as a protected band within the solvent plug at the start of the chromatogram. When breakthrough occurs, the effective concentration of sample that is retained under conventional chromatographic conditions is lowered. This can be a problem when insensitive detection methods (such as NMR) are used, because sample concentrations need to be as high as possible. Another possible issue with breakthrough is the non-representative splitting of the sample, i.e. the portion of the sample that is eluted as an unretained peak and the portion of the sample that is eluted under conventional chromatographic conditions are not equivalent (24). Quantitation is also not possible when breakthrough occurs. In general, it is best to avoid breakthrough effects if possible. Other overloading effects, such as band broadening and fronting or tailing, should also be avoided since these will jeopardise the quality of the separation.

Detector-cell volume

Detection cells in NMR are much larger than those used in other LC detectors (between 40 and 120 µL, compared to typically 8 µL in a UV detector cell). This will lead to a loss of resolution between chromatographic peaks. The increased cell volume is necessary not only to increase the amount of sample in the cell at any one time, but also to ensure that the residence time of a molecule in the detector cell (Ta) is long enough. Ta is defined as the ratio between the detection volume of the flow cell and the flow rate of the mobile phase through the cell. Low Ta values (lower than 5 seconds) lead to reduced NMR spectral resolution (16). In order to maximise residence times, flow-
rates for LC-NMR are usually lower than normal. An optimal balance must be struck between maximising residence times and minimising diffusion and extra-column band broadening of the separated peaks. In this respect, polymeric samples are usually easier to work with, because the diffusion coefficients of macromolecules are significantly lower than those for smaller molecules. Flow-rates as low as 0.2 mL/minute on 8 x 300 mm columns have been reported (20).

**Experimental**

**Samples**
Styrene-methylmethacrylate copolymeric standards with narrow and well-defined chemical-composition distributions were obtained from the polymer-chemistry group of the Technical University of Eindhoven (TU/e), The Netherlands. Copolymers with a bimodal chemical-composition distribution (styrene-methylmethacrylate) were also obtained from TU/e. Both the standards and the sample polymers had random sequences. Block copolymers of poly(styrene-co-methyl methacrylate) were obtained from Polymer Standard Services (Mainz, Germany).

**HPLC**

Chromatographic separations were carried out on an Agilent 1100 liquid chromatograph using UV detection at 260nm. Sample concentrations were approximately 20 mg/mL and the injection volume was 250 µL. The stationary phase was Discovery C18 from Supelco (4.6 x 150 mm, 5 µm) (Bellefonte, PA). The mobile phase was a mixture of dichloromethane and acetonitrile. These solvents were chosen because they have relatively straightforward NMR spectra. Both were LiChrosolve HPLC grade from Merck (Darmstadt, Germany). The flow rate was 0.4 mL/minute. Separations were carried out at ambient temperature.

**HPLC-NMR**

A Bruker AMX 600 spectrometer was connected to the LC system via a Bruker BSFU interface using a stainless-steel capillary of approximately 3 m. The volume of the flow probe of the NMR was 120 µL. To stabilise the lock, 15% deuterated acetonitrile was added to the acetonitrile mobile phase. HPLC-NMR measurements were recorded at 298 K using a solvent presaturation with shaped pulses for solvent suppression (lc2pnps). A total of either 128 or 256 rows were measured in the F1 (time) dimension. Each row had 169 transients. Both dimensions (time and chemical shift) were multiplied by a squared sine function before Fourier transformation.
Results and Discussion

LC separation of the copolymers

The LC experimental conditions were optimised for separation according to chemical composition using copolymeric standards. Figure 1 shows the mobile-phase composition at the point of elution for a series of poly(styrene-co-methyl methacrylate) standards. PMMA homopolymer elutes the earliest and free polystyrene is retained until the mobile phase reaches approximately 60% DCM. The copolymers are eluted in the window between the two homopolymers, the higher the fraction of styrene in the copolymer, the more that copolymer will be retained.

![Graph showing elution composition of the mobile phase for a series of styrene/MMA copolymers standards](image)

**Figure 1:** Elution composition of the mobile phase for a series of styrene/MMA copolymers standards (see chapter 2 also).

The random PS-PMMA bimodally distributed copolymeric samples (samples 1 to 3) were separated using the same experimental conditions as for the standards, except that in each case, the gradient program was optimised to suit the particular sample, i.e. to maximise the separation according to chemical composition.

Figures 2 to 4 show the gradient-LC separations of the three random copolymers according to their bimodal chemical composition. In each case, two main peaks are seen, corresponding to the bimodal chemical-composition distributions present in each of the samples. An unretained peak due to ‘breakthrough’ can also be seen at the start of the run. Some free PMMA homopolymer may also elute at this point. However, this cannot be seen by the UV detector. The last peak in the chromatogram corresponds to free PS homopolymer.
Figure 2: Sample 1. Randomly distributed copoly(styrene-methylmethacrylate), average composition = 44% styrene. 200 µL injection volume. 30 to 80% DCM in ACN in 90 minutes. Solvent suppression at 38.6% DCM.

Figure 3: Sample 2. Randomly distributed copoly(styrene-methylmethacrylate), average composition = 58% styrene. 250 µL injection volume. 30 to 80% DCM in ACN in 90 minutes. Solvent suppression at 35.5% DCM.
Figure 4: Sample 3. Randomly distributed copoly(styrene-methylmethacrylate), average composition = 50 %
Styrene. 250 μL injection volume. 40 to 70% DCM in ACN in 120 minutes. Solvent suppression at
45.8% DCM.

The area under each of the copolymeric peaks is only due to the concentration of polystyrene
eluting at that time, rather than the concentration of the copolymer itself (because PMMA does not
absorb in the UV region). Therefore no information on the composition of the copolymer, i.e. the
ratio of styrene to methylmethacrylate, or the concentration of the copolymer at any point in the
chromatogram can be obtained from the UV detector. For the direct determination of the chemical-
composition distribution(s) of a sample, the separation must be coupled to a detector such as NMR
that can measure the relative concentration of each of the two monomers present in the copolymer.

Solvent suppression

In order to minimise the overwhelming NMR signals due to the protons in non-deuterated mobile-
phase solvents, solvent-suppression techniques are required. When gradient separations are
performed, changes in the solvent composition over the gradient run can complicate solvent
suppression. Either an automated, continuously-optimising solvent suppression must be applied
along the gradient or a single optimal solvent suppression performed (optimised at an average
mobile-phase composition, usually corresponding to the composition around the maximum of the
peak of interest). In our case, we used shaped pulses for solvent suppression, taking the average
mobile-phase composition of the eluting peak as the optimised composition. Some deuterated

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acetonitrile (15% vol./vol.) was required to establish a solvent lock, but this did not affect the quality of the LC separation.

NMR data interpretation

The ratio of styrene to methylmethacrylate present in the copolymer was calculated from the ratio of signals due to the aromatic protons of the styrene monomer (at around 7 ppm) and to the methyl ester protons on the MMA monomer (around 3.5 ppm). Since there are five aromatic protons in a styrene monomer and three methyl-ester protons in a MMA monomer, a signal ratio of 5:3 (aromatic:methyl ester protons) corresponds to a polymer that contains 50% styrene and 50% methyl methacrylate. The Examples of the NMR spectra obtained for each of the three random copolymers at different times in the chromatogram are shown in Figures 5, 6 and 7.
Figure 5: Sample 1. NMR spectra taken at different times in the chromatographic run. (a) 25.5 minutes (b) 46 minutes (c) 50 minutes.
Figure 6: Sample 2. NMR spectra taken at different times in the chromatographic run. (a) 22.5 minutes (b) 46.5 minutes (c) 53 minutes.
Figure 7: Sample 3. NMR spectra taken at different times in the chromatographic run. (a) 25 minutes (b) 40 minutes (c) 49 minutes.
In most cases, the aromatic signal is clearly seen as a broad peak around 7 ppm. Once the signal-to-noise ratio is sufficiently high, quantitation of this signal is reasonably straightforward. The signal due to the methyl-ester protons of the MMA monomer is more difficult to quantify, because there is significant broadening of the peak. The broadening is due in part to the different environments of the randomly distributed MMA. However, the extent of the broadening is much more significant than might be expected and is not yet fully understood. The broader signal means that the signal-to-noise ratio for a given concentration of sample is dramatically reduced. An accurate quantitation of the area under the peak for the methyl-ester protons was not always possible, especially at the leading and tailing edges of the peaks where the concentration was at its lowest. Solvent suppression of the proton signals due to acetonitrile in the mobile phase adds to the problem, because the phase correction that is required for solvent suppression affects the baseline in the region close to the methyl-ester proton signal.

The results from the integration of the three randomly distributed copolymers are shown graphically in figures 8, 9 and 10, along with the corresponding chromatograms. In each case the concentration of styrene in the polymer should increase with increasing time (increasing solvent strength in the mobile phase). It would therefore be expected that the NMR results would show that the fraction of PS in the polymer increases with increasing time. The results from the NMR data indicate a general trend of the concentration of styrene in the eluting polymer increasing with increasing retention time. However, the data are very scattered and it is not possible to fit the points to a curve that can accurately correlate retention time with chemical composition (for the determination of the CCD). This is the case for each of the three random copolymers.

![Figure 8: Sample 1. Calculated change in composition according to NMR signals across the chromatographic peaks.](image-url)
Sample 2. Calculated change in composition according to NMR signals across the chromatographic peaks.

Sample 3. Calculated change in composition according to NMR signals across the chromatographic peaks.

The problem with the data is that the signal due to the methyl ester protons of PMMA is too low to be accurately quantified. For an accurate determination of the CCD using LC coupled online to NMR, the concentration of the polymer in the detector cell must be increased so that the signal-to-noise-ratio is high enough for accurate quantitation. This can be achieved by increasing the sample concentration or the sample volume injected into the system or by increasing the residence time of the sample in the detector cell (by decreasing the flow-rate). Another option is to take fractions across the peaks and to measure the fractions in the batch mode. In this case, each fraction could be
measured for as long as required for an acceptable signal to noise ratio. Solvent-suppression techniques would not be required, because the samples could be re-dissolved in a deuterated solvent. A disadvantage of this approach is that some chromatographic resolution would be lost due to fractionation.

Determination of the block-length distribution of block polystyrene-methylmethacrylate copolymers

Three block copolymers were separated according to chemical composition to characterise their block-length distributions (BLD). Once again, the gradient programs were optimised for the particular sample, i.e. to maximise the separation according to chemical composition. For the block copolymers, breakthrough of the sample was a more-significant problem than for the random copolymers and large unretained peaks were observed.

LC-NMR coupling

Solvent suppression was optimised at an average mobile-phase concentration (in this case the average mobile-phase composition of the main eluting peak). Examples of the NMR spectra obtained for the three block-copolymer samples (samples 4 to 6) are given in figures 11, 12 and 13. The methyl ester proton signal was much sharper for the block copolymer than for the random copolymer. This is due to the structured nature of the block copolymer (i.e. every MMA monomer is linked to two other MMA monomers except the MMA linked to the styrene block). The environment of the methyl ester protons in this case is much more homogeneous resulting in a significantly sharper signal. This increases the signal-to-noise ratio of the methyl ester proton signal at a given concentration, allowing much more accurate integration of the peak.
Figure 11: Sample 4. Block copoly(styrene-methylmethacrylate), NMR spectra taken at different times in the chromatographic run. (a) 12 minutes (b) 19.5 minutes (c) 30 minutes.
Figure 12: Sample 5. Block copoly(styrene-methylmethacrylate). NMR spectra taken at different times in the chromatographic run. (a) 17 minutes (b) 23.5 minutes (c) 28 minutes.
Figure 13: Sample 6. Block copoly(styrene-methylmethacrylate), NMR spectra taken at different times in the chromatographic run. (a) 35.5 minutes (b) 55 minutes (c) 66 minutes.
Figures 14, 15 and 16 show the chromatograms of each of the three block copolymers and the calculated ratio of styrene to MMA in the polymer at points across the chromatogram. In comparison to the random copolymeric samples, the trend towards increased styrene content over time is much clearer for the block copolymers. Noted exceptions are at the beginning and end of the peak, where the concentration of sample was too low for accurate quantitation. However, even for the block copolymers, where the methyl-ester-proton signal is much sharper, a significant amount of scatter due to inaccuracies in the quantitation of the two peaks is present and it is doubtful that an accurate determination of the BLD could be calculated.

Figure 14: Sample 4. Average composition = 44% styrene. 200 µL injection volume. 40 to 60% DCM in ACN in 60 minutes. Solvent suppression at 44% DCM. Points correspond to the calculated change in composition according to NMR signals across the chromatographic peaks.
Figure 15: Sample 5. Average composition = 48% Styrene. 150 µL injection volume. 50 to 65% DCM in ACN in 45 minutes. Solvent suppression at 54.2% DCM. Points correspond to the calculated change in composition according to NMR signals across the chromatographic peaks.

Figure 16: Sample 6. Average composition = 65% Styrene. 200 µL injection volume. 50 to 65% DCM in ACN in 90 minutes. Solvent suppression at 56.2% DCM. Points correspond to the calculated change in composition according to NMR signals across the chromatographic peaks.
Conclusions

The direct determination of the chemical composition of random and block polystyrene-methyl methacrylate showed some promise using LC coupled on-line to NMR. Some further optimisation of the experimental set-up is required, in order to improve the signal to noise ratio of the NMR spectrometers. This could be achieved by (a) minimising sample loss due to chromatographic breakthrough (b) increasing the amount of sample that is injected (scaling up of the LC system may be required), (c) increasing the residence time of the analyte molecules in the probe by decreasing the flow rate, (d) taking fractions across the chromatographic peaks and measuring in the off-line mode.

The difference in the broadness of the methyl ester signal of the randomly distributed and block copolymers is larger than expected and further investigation may be required for a fuller explanation. If a clear relationship between the width of the signal and the structure of the polymer (random to block) can be established, then this could be used to quantify the blocky character of the samples. Some work has previously been reported on this subject (21).

References

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