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Co-Polymer sequence determination over the molar mass distribution by size-exclusion chromatography combined with pyrolysis - gas chromatography

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ABSTRACT

The chain sequence of co-polymers strongly affects their physical properties. It is, therefore, of crucial importance for the development and final properties of novel materials. Currently however, few analytical methods are available to monitor the sequence of copolymers. The currently preferred method in copolymer-sequence determination, nuclear-magnetic-resonance spectroscopy (NMR), is insensitive (especially when ¹³C-NMR is required) and often offers little spectral resolution between signals indicative of different subunits. These limitations are especially challenging when one is interested in monitoring the sequence across the molar-mass distribution or in quantifying low abundant subunits. Therefore, we set out to investigate pyrolysis - gas chromatography (Py-GC) as an alternative method. Py-GC is more sensitive than NMR and offers better resolution between various subunits, but it does require calibration, since the method is not absolute. We devised a method to fuse data from NMR and Py-GC to obtain quantitative information on chain sequence and composition for a set of random and block poly(methyl methacrylate-co-styrene) copolymer samples, which are challenging to analyse as MMA tends to fully depolymerize. We demonstrated that the method can be successfully used to determine the chain sequence of both random and block copolymers. Furthermore, we managed to apply Py-GC to monitor the sequence of a random and a block copolymer across the molar-mass distribution.

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1. Introduction

The sequence of co-polymers, *i.e.* the order in which monomers are incorporated in the polymer backbone, strongly affects their physical properties. Therefore, the ability to characterize the sequence distribution is crucially important for the development and final properties of novel materials [1–4]. Modern living / controlled polymerization methods, including, for example, nitroxide-mediated radical polymerization (NMP), reversible addition-fragmentation chain transfer (RAFT) and atom-transfer radical polymerization (ATRP), allow chemists to assume control

of the polymerization and thus of the resulting architecture of the polymer [5–9]. Validation of synthesis results does, however, require suitable analytics. Essentially, the co-polymer chain sequence – and thus the degree of randomness of monomer incorporation – can vary between two extremes, *viz.* block (AAABBB) and alternating (ABABAB). Schematic examples of typical co-polymer sequences can be found in Fig. 1.

The sequence arises from the reaction kinetics during synthesis, which depend on the monomer feed ratios and reactivities [10]. If the reactivity ratios are equal between the different monomers the sequence will have a random character. If the ratios differ significantly, blocky or alternating polymers will form [10,11]. In analogy with molar-mass averages as determined using size-exclusion chromatography (SEC), the sequence distribution of

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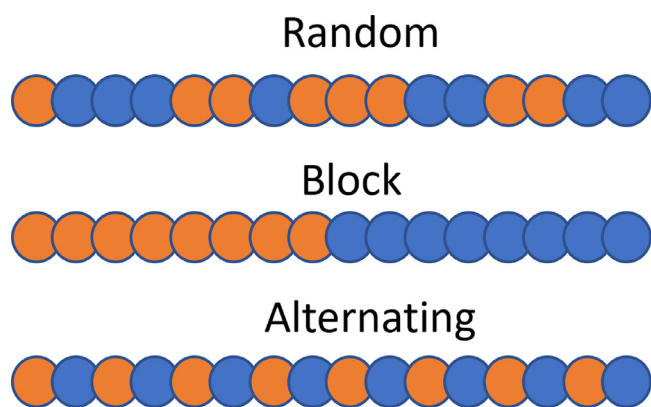


Fig. 1. Schematic representation of random, block and alternating co-polymers.

co-monomers in the chain can be expressed as the number-average sequence length (NASL), which is defined as the average number of monomer units across all blocks of a specific monomer in a polymer. The NASL can be quantified from the analysis of subunits in the polymer, such as dimeric units (“diads”; Eqs. (1a) and (1b)) and triads, as shown in the Eqs. (2a) and (2b) below [12,13].

$$N_A = \frac{n_{AA} + \frac{1}{2}n_{AB+BA}}{\frac{1}{2}n_{AB+BA}} \quad (1a)$$

$$N_B = \frac{n_{BB} + \frac{1}{2}n_{AB+BA}}{\frac{1}{2}n_{AB+BA}} \quad (1b)$$

$$N_A = \frac{n_{AAA} + n_{AAB+BAA} + n_{BAB}}{\frac{1}{2}n_{AAB+BAA} + n_{BAB}} \quad (2a)$$

$$N_B = \frac{n_{BBB} + n_{BBA+ABB} + n_{ABA}}{\frac{1}{2}n_{BBA+ABB} + n_{ABA}} \quad (2b)$$

In which N_A and N_B indicate the NASL of the A and B monomer respectively and n_x is the molar fraction of the respective triad or diad in the polymer. If the NASL of both monomers is known, then the degree of randomness (R) can be calculated using Eq. (3).

$$R = \frac{1}{N_A} + \frac{1}{N_B} \quad (3)$$

The degree of randomness of an ideal random co-polymer equals 1. When the polymer has an alternating tendency the degree of randomness increases to a maximum of 2, whereas R will approach 0 when the polymer is a block-copolymer or the sample is a polymer blend. In some cases, including the example presented in this work, only part of the theoretically present subunit species can be determined experimentally. Additionally, if the composition of the co-polymer is known then the NASL of one monomer can be used to calculate the NASL of the other as shown in Eq. (4) [14].

$$N_A = \frac{N_B - x_B N_B}{x_B} \quad (4)$$

In which x_B indicates the molar fraction of monomer B in the co-polymer. To perform these calculations experimental measurement of the concentration of subunits (i.e. diads or triads) in the polymer backbone is required. While the degree of randomness can be modelled based on reaction kinetics using various models, experimental methods for parametrization and validation are always needed for confirmation [15]. The sequence is notoriously hard to determine, as it requires quantitative measurements of the numbers of subunits in the chain (i.e. diads or triads), which can only be achieved by a limited number of methods [13,16–18]

Methods to study the sequence of co-polymers include infrared spectroscopy [17,19], mass spectrometry (MS) [20–22], pyrolysis – gas chromatography (Py-GC) [23] and nuclear-magnetic-resonance spectroscopy (NMR) [12,13,16,24–26]. Despite the availability of various methods, co-polymer sequence determination is rarely straightforward. MS is typically limited to low-molar-mass polymers, while infrared spectroscopy usually offers insufficient resolution to determine the sequence of co-polymers. NMR is the most commonly used method for the sequence determination of co-polymers by quantification of di- and triads or larger subunits [12,16,24–26]. However, sequence determination by NMR is not always straightforward, as signals related to specific subunits (i.e. triads) tend to overlap [27]. ^{13}C -NMR is often needed to distinguish between different subunits. However, its poor sensitivity renders sequence determination even more challenging [13]. Another aspect to consider when performing sequence determination with NMR is that the correlations between different nuclei typically only stretch across a limited number of bonds (≤ 8) [28]. This limits the application of NMR to the sequence determination of relatively simple co-polymers consisting of a few monomers (binary or tertiary copolymers), with diads or triads having fewer than eight carbons in the backbone. The presence of hetero atoms in the chain may limit this further [28]. A further, and in the authors' view even more-serious disadvantage of the abovementioned approaches is that the microstructural parameters obtained constitute mere averages of complex distributions. Due to non-ideal polymerization conditions, the average of any kind of structural parameter is never a complete descriptor of the real polymer composition [29]. In addition to the NASL of the whole polymer, its variation along, for example, the molar mass distribution (MMD) is of great interest. Krämer et al. hyphenated SEC to ^1H -NMR to study the NASL of polymers as a function of their MMD, gaining a deeper insight in the heterogeneity of the sample [30]. While LC-NMR is a powerful tool, as shown by Krämer et al., it is challenging to implement and requires extensive optimization. Furthermore, the sequence cannot always be determined by ^1H -NMR. Often ^{13}C -NMR is required, the lower sensitivity of which is incompatible with online monitoring of LC effluents. Even for online compositional analysis, i.e. for determining the ratio of co-monomers, LC-NMR is still rarely applied in analytical laboratories, due to the high complexity and cost of equipment, limitations of solvent-suppression techniques, and the overall lack of sensitivity [31].

Pyrolysis-GC is a long-established alternative method to determine the sequence of co-polymers [32,33]. Wang et al. demonstrated its use on a variety of co-polymers, such as styrene-methyl methacrylate (S-MMA), styrene-butyl acrylate and (partially) chlorinated polyethylene [14,23,34]. The results were in agreement with NMR results, rendering py-GC a possible alternative for sequence determination. Unlike NMR, py-GC is reliant on calibration (e.g. by suitable reference measurements). However, it offers other advantages. py-GC offers a much higher sensitivity (i.e. much lower detection limits) for most polymer-classes and often a significantly better chemical (isomer) resolution between differing diads and triads compared to NMR. While multiple researchers have seen the added value of hyphenating LC to py-GC for compositional analysis [35,36]. The increased sensitivity of py-GC is especially an advantage when one is interested in determining polymer microstructure or sequence across the MMD by coupling with LC.

In the current work, we aimed to investigate the use of py-GC for determining the polymer sequence heterogeneity as a function of molar mass and to apply the technique to a variety of S-MMA random and block co-polymers. Our objective was to obtain in-depth information on the chemical nature of the polymers by a combination several methods. We aimed to determine the co-polymer composition and to characterize the monomer sequence

Table 1
Synthesis recipes of studied samples.

Sample	Sequence	Synthesis recipe ratio styrene/MMA (%w/w)
1	statistical	25/75
2	statistical	50/50
3	statistical	75/25
4	block	25/75
5	block	50/50
6	block	75/25

across the MMD by coupling SEC to refractive-index and UV/VIS detectors, as well as to py-GC.

2. Materials and methods

2.1. Sample synthesis

Reference polymers were synthesized using free-radical polymerization. Styrene (Honeywell, Morristown, NJ, USA) and methyl methacrylate (Sigma Aldrich, Darmstadt, Germany) monomers were mixed in various ratios (by weight). The total mass of monomers was kept constant at 2 g. Then 6 mL of a 3 g/L 2,2'-azobis(2-methylpropionitrile) (AIBN; Sigma Aldrich) solution in butanone (Sigma Aldrich) was added. The samples were incubated at 70°C overnight (16 h) under gentle stirring in a water bath. Samples were cooled to room temperature and the polymer fraction was precipitated by addition of two volumes methanol, decanted, and dried in a vacuum oven overnight at 70°C and 200 mbar. The block co-polymers were supplied by Covestro (Leverkusen, Germany) and were synthesized as follows. Styrene and MMA (Dow Chemical, Terneuzen, The Netherlands) were polymerized in toluene at 90°C using Perkadox 2,2'-Azodi(2-methylbutyronitrile) (AMBN; Akzo Nobel, Deventer, The Netherlands) as an initiator and 4-cyano-4-(((dodecylthio)carbonothioyl)thio)pentanoic acid (CSIRO, Canberra, Australia) as RAFT agent. Conversions were monitored by py-GC as described in ref [37]. Target styrene percentages in the copolymers were 25, 50 and 75% for block samples 4, 5 and 6 (Table 1), respectively.

2.2. Size-exclusion chromatography and fractionation

An Agilent (Waldbronn, Germany) 1200 series LC system was used with an Agilent 1200 fraction collector. The system was coupled to an Agilent 1260 refractive index detector (RID) and a 1260 Infinity II UV detector. SEC was performed with pure tetrahydrofuran (THF, Bernd Kraft isocratic grade, Duisburg, Germany), using three PLgel MIXED-B columns (300 × 7.5 mm i.d., 10 μm particle size; Agilent) at a flowrate of 1 mL/min. The MMD and composition were determined based on 50 μL injections of 2 mg/mL of sample. Calibration of the MMD was achieved using an Easi-Cal PS standard mix (Agilent). WinGPC software (PSS, Mainz, Germany) was used to analyse and export the data. Data evaluation of the RID- and UV-chromatograms was achieved by a homemade MATLAB script (Mathworks, Natick, MA, USA). The inter-detector volume was determined using narrowly distributed PS standards. For the fractionation, samples 2 and 5 (Table 1) were dissolved at a concentration of 5 mg/mL in THF (Honeywell, Morristown, NJ, USA) 100 μL were injected and fractionated into 13 fractions of 0.5 min each, eluted between 19 and 25.5 min. Fractionation was repeated three times and the fractions were pooled, dried in a vacuum oven overnight at 70°C and 200 mbar under nitrogen, and re-dissolved in a desired volume of THF prior to py-GC analysis.

2.3. GC-analysis

A 2010 Shimadzu heart-cut two-dimensional gas chromatography-MS (GC-GC-MS) instrument ('s-Hertogenbosch, The Netherlands) operated in 1D mode with an OPTIC-4 PTV (GL Sciences, Eindhoven, The Netherlands) was used for the analysis of the samples and fractions. A column with DB-5 stationary phase (30 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent) was used. Helium at a column flowrate of 1.4 mL/min was used and a split of 1:50 was employed. The temperature program was 40°C for 5 min, then at 20°C/min to 320°C and held at 320°C for 3 min. The samples were injected in a sintered liner (GL Sciences) at 40°C. After a delay of 5 s the PTV was heated at 60°C/s to 120°C, held at 120°C for 1 min, after which the temperature was further increased at 60°C/min to 500°C to pyrolyze the sample. After pyrolysis for 1 min the PTV was cooled to 300°C for the rest of the run. The MS was operated in scan mode ($m/z = 40-400$) with a 0.3 s event time and 70 eV ionization voltage. The source temperature and the interface temperature were 200°C and 280°C respectively.

2.4. NMR

Measurements were performed in deuterated chloroform ($CDCl_3$, Sigma) on a Bruker (Rheinstetten, Germany) Avance NEO 500 MHz NMR spectrometer at 298 K. Approximately 10 mg of the block copolymer or 50 mg of a random co-polymer were dissolved in 0.7 mL $CDCl_3$ (Sigma). A total of 32 scans were recorded for the 1H measurements with an acquisition time of 2.7 seconds and a pulse delay of 5 s. The digital resolution was 0.37 Hz and the flip angle was 90°.

The proton-decoupled ^{13}C -NMR spectra were recorded on the same instrument at 300 K. A total of 8192 scans were recorded with a pulse delay of 4 s and an acquisition time of 1.09 s. The digital resolution was 0.92 Hz with a flip angle of 90°.

3. Results and discussion

To investigate the feasibility of SEC-py-GC to determine the chain sequence of polymers, it is imperative that representative and well-characterized polymer samples are used. Therefore, random and block S-MMA copolymers with varying chemical compositions were synthesized (see Table 1).

3.1. Chemical composition and chemical composition heterogeneity determination

The composition of the synthesised samples was determined using 1H -NMR measurements (Table 2). The ratio of the total signal intensities of the aromatic and aliphatic regions was used to determine the chemical composition, as described by Aerdts et al. [27,38].

In determining the sequence of S-MMA co-polymers by py-GC, direct calculation of the MMA block length (Eq. (1)) is not possible, since MMA does not form diads in pyrolysis. Therefore, knowledge of the composition as a function of molar mass is required. SEC-UV-RID was performed on co-polymer samples to determine the MMD and chemical composition drift across the MMD. The results are shown in Fig. 2. The chemical composition was calculated using Eq. (5) [39].

$$\frac{1}{W_s} = 1 - \frac{(Z * f_{S, RID}) - f_{S, UV}}{(Z * f_{M, RID}) - f_{M, UV}} \quad Z = \frac{S_{UV}}{S_{RID}} \quad (5)$$

In which W_s is the weight fraction of styrene, Z is ratio between the UV and RID signal (S_{UV} , S_{RID}), $f_{S, UV}$ and $f_{M, UV}$ are the UV response factors for styrene and MMA, respectively. The RID response

Table 2

Chemical composition of various samples obtained by SEC-UV-RID, py-GC (calibrated by $^1\text{H-NMR}$) and $^1\text{H-NMR}$ as well as the MMD and PDI determined by SEC-UV-RID.

Sample	Composition mole-% styrene NMR	Composition mole-% styrene SEC-UV-RID	Composition mole-% styrene py-GC	M_z (Da)	PDI
1	25.8	28.5	27.9	39350	1.62
2	45.6	49.5	45.6	33520	1.63
3	67.4	73.2	66.4	26730	1.68
4	23.5	28.9	25.3	27660	1.28
5	52.8	56.9	50.3	24580	1.34
6	80.2	83.8	79.6	20940	1.34

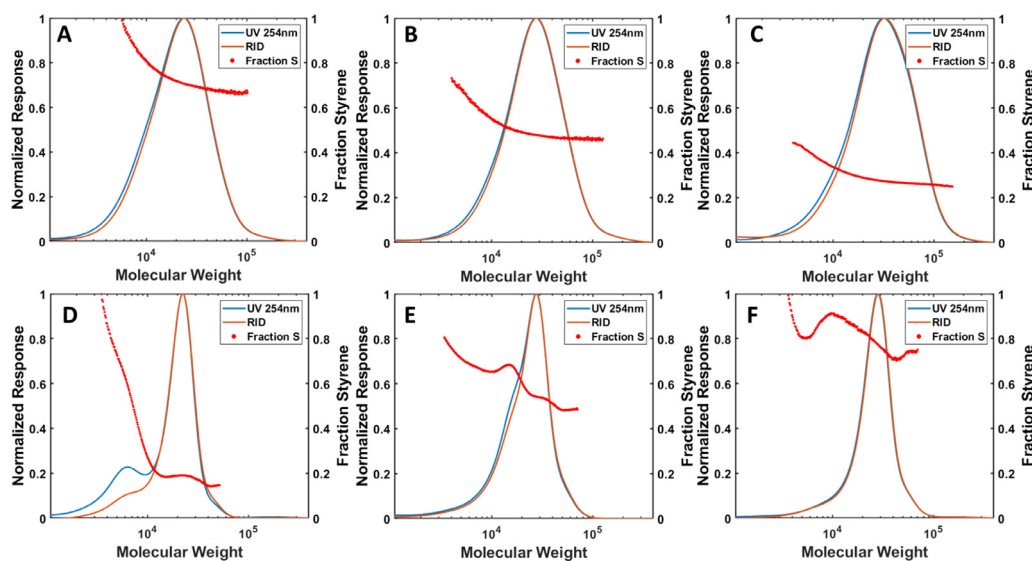


Fig. 2. MWDs determined from SEC chromatograms (left-hand axis) of three random co-polymers 1-3 (A-C) and three block co-polymers 3-6 (D-F). The composition determined by detection with UV and RID is overlaid in red (right-hand axis). Conditions are outlined in the Experimental section.

factors for styrene and MMA are denoted as $f_{S, RID}$ and $f_{M, RID}$, respectively. The response factors applied in the chemical composition calculation with SEC-UV-RID were determined based on homopolymers, synthesised using the same method as the random co-polymers.

The results in Fig. 2 suggest that the chemical composition of the co-polymers is heterogenous, especially at the high and low molar mass-ranges of the distributions. However, it should be noted that in these regions, where the concentrations are low, the method becomes less reliable, as small errors in background correction and the determined inter-detection volume can lead to large errors in the determined composition. Furthermore, the band broadening between the UV and RI detectors can impact this procedure in case of steep signal gradients, even in chromatographic regions with high signal-to-noise ratio. Nevertheless, the determined weight fraction of styrene appeared to increase sharply in the low-MM fractions of the samples. This could in part be explained by the presence of a free styrene block (homopolymer) in the block copolymers, the presence of which could be confirmed by interaction LC studies [40,41]. Another explanation for the composition change in the lower MM region (< 10 kDa) could be a change in the RID response factor in the low MM region, although this seems unlikely as this effect is typically insignificant above a MM of 5 kDa [42]. The high styrene content at the low-MM end is more-likely induced by UV active end-groups, especially in the samples synthesised with the RAFT polymerization (samples 4-6). The chromophores of these end groups cause additional UV absorption at 254 nm. This is supported by the overall composition of the co-polymers determined by SEC-UV-RID (Table 2), with the styrene content being higher than the composition determined by $^1\text{H-NMR}$.

Since the composition determined by SEC-UV-RID is less reliable in the lower MM region, an attempt was made to use the py-GC results for a determination of composition. A mass-balance approach was used to calculate the composition with py-GC. The total area for styrene and MMA was based on the assigned diads and triads in the pyrogram, weighted by their molar content of the respective monomer (see Section 3.2 for more details on the assignment), as described in Eqs. (6)-(8).

$$S_{\text{total}} = A_s + A_{ss} + \frac{1}{2}A_{sm} + \frac{2}{3}A_{ssm+mss+sms} + A_{sss} \quad (6)$$

$$M_{\text{total}} = A_m + \frac{1}{2}A_{sm} + \frac{1}{3}A_{ssm+mss+sms} \quad (7)$$

$$\% \text{Styrene} = \frac{S_{\text{total}}}{S_{\text{total}} + (M_{\text{total}} \cdot F_M)} \quad (8)$$

Here, S_{total} denotes the total styrene area, and M_{total} the MMA area. A_x denotes the peak area of the various observed diad and triad peaks. F_M is a correction factor for differences in detection sensitivities, calculated based on the NMR results by minimizing the sum of the absolute difference between the overall compositions calculated from $^1\text{H-NMR}$ and from py-GC results. In the present case, F_M was calculated to be 1.80. The resulting correlation to NMR can be seen in Fig. 3, without the calculated correction factor ($F_M = 1$) and with. Both the random (samples 1-3, Table 1) and block (samples 4-6, Table 1) co-polymers show good correlation with the NMR data using the devised mass-balance approach.

The F_M -corrected values for the composition determined with py-GC are listed in Table 2. The data shows good correlation with the NMR results after calibration, for both the block- and random

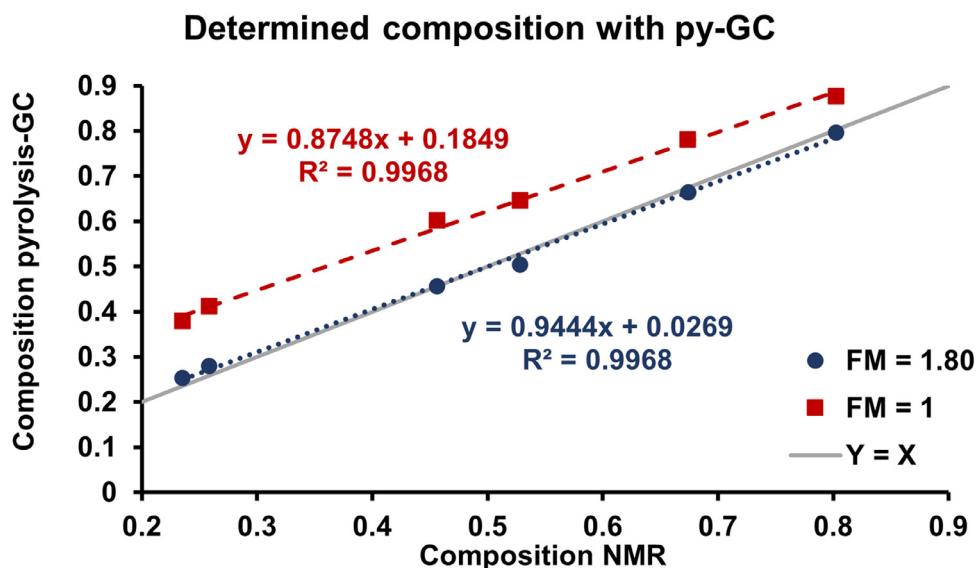


Fig. 3. Correlation between composition determined by $^1\text{H-NMR}$ and by using the mass-balance approach outlined in Eqs. (6)–(8), with and without a sensitivity correction, i.e. $F_M = 1.80$ (blue) and $F_M = 1$ (red), respectively. The grey line indicates the $y = x$ line.

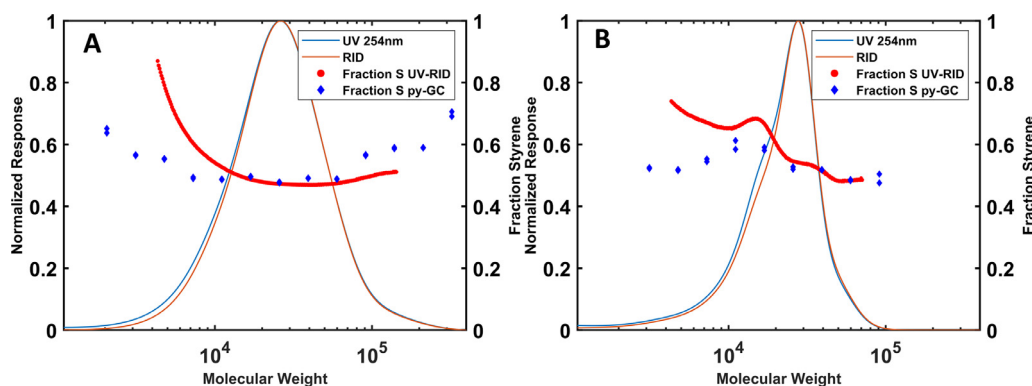


Fig. 4. MWDs determined from SEC chromatograms of a statistical co-polymer (sample 2, Fig. 4A) and a block-copolymer (sample 5, Fig. 4B) with the composition determined by SEC-UV-RID overlaid in red and the composition calculated from py-GC data (with NMR calibration) indicated as blue diamonds. Conditions are outlined in the Experimental section.

-copolymers, suggesting that the method may be more suitable than SEC-UV-RID for the determination of the composition across the MMD Fig. 4. features a comparison between the composition across the MMD (i) calculated based on the pyrolysis of the fractionated samples with calibration factors determined by NMR and (ii) determined by SEC-UV-RID.

As can be seen in Fig. 4, the increase in styrene percentage in the low MM region that is observed with SEC-UV-RID is alleviated by the direct quantification approach. The fraction of styrene determined by both methods is equal at the peak maximum, indicating a good general agreement. In the SEC-UV-RID method the determination of the baseline and the inter-detector volume may be too critical to yield accurate results at the MM extremes, where concentrations are low. It can thus be concluded that py-GC (calibrated by NMR) is found to be a more suitable alternative for determining chemical composition heterogeneity of the fractionated samples than SEC-UV-RID in this study.

It should be mentioned that the composition was only required as a supplement to py-GC data as the NASL of MMA can only be determined indirectly using the overall composition and Eq. (4). This is the case since MMA containing oligomers tend to depolymerize to monomers during pyrolysis, so that diad and triad MMA fragments are not observed.

3.2. Sequence determination by py-GC

To evaluate the suitability of the method to determine the NASL of the different polymers, all samples were subjected to py-GC-MS analysis. The random co-polymers (samples 1–3, Table 1) were also sequenced by NMR to establish calibration factors for py-GC. In Figs. 5A and 5B, the pyrograms can be found of a statistical copolymer (sample 2) and a block copolymer (sample 5), respectively. Even at first glance, the difference in the ratio between the styrene and hybrid diad in the pyrograms clearly indicates a more block-like microstructure for this co-polymer.

As shown by Wang et al. [14], py-GC can be applied to determine the average styrene block length from the SS and SM diads only. To convert the diad peak areas to an average sequence length Eq. (1a) is applied, yielding NASL values for styrene. However, since py-GC is not quantitative in an absolute sense, the determined values will have to be calibrated, $^{13}\text{C-NMR}$ was applied to the random co-polymers according to a method described by Aerdts et al. [27]. The sequence values and randomness calculated using this method are reported in Fig. 6. The degrees of randomness (R) obtained with $^{13}\text{C-NMR}$ are relatively constant across the MMD of the samples.

Although the $^{13}\text{C-NMR}$ spectral assignments of styrene-MMA co-polymers have been a topic of debate [27,38], Wang et al. re-

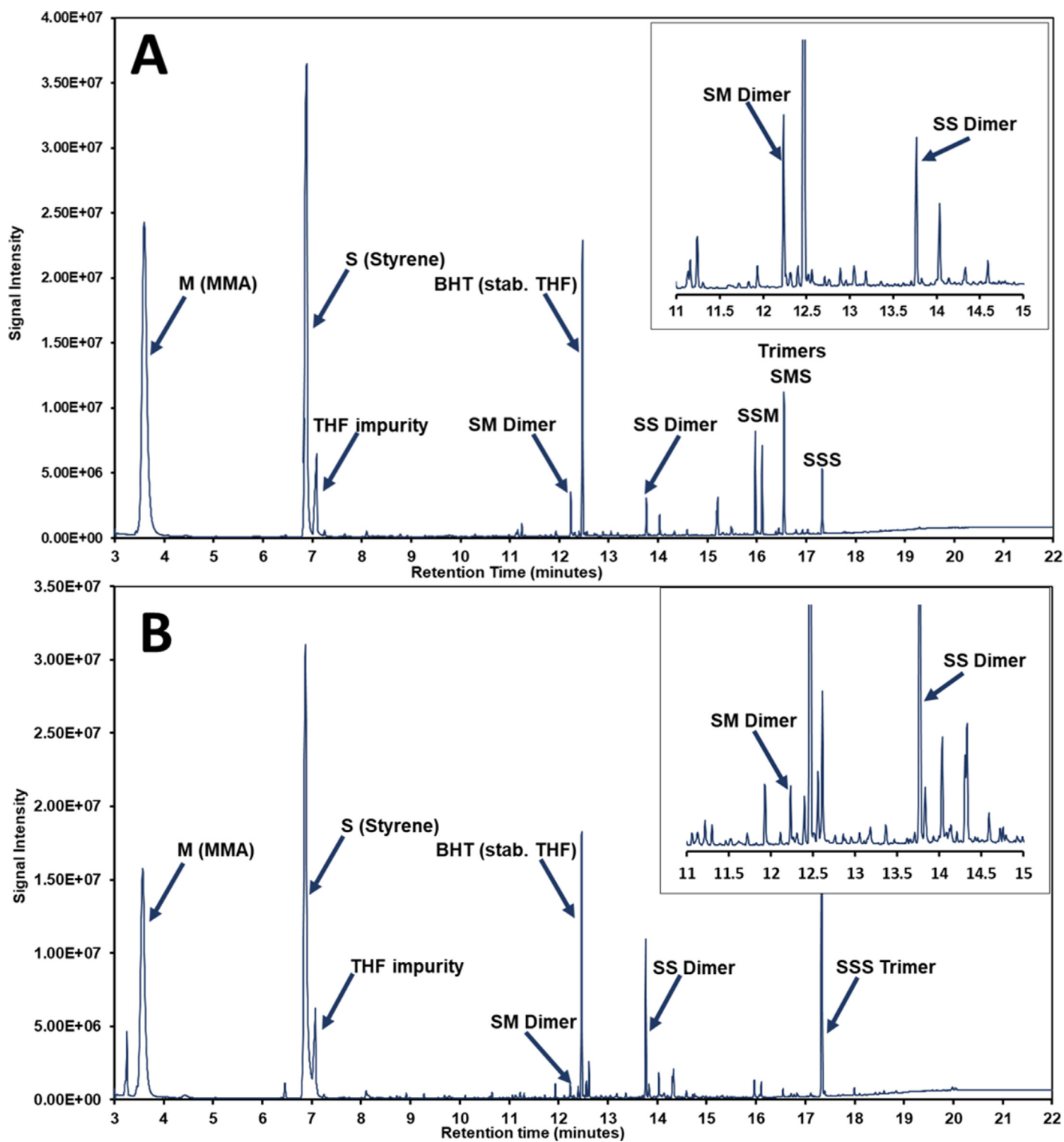


Fig. 5. Example pyrograms of a S-MMA random co-polymer (A; sample 2, Table 1) and a S-MMA block co-polymer (B; sample 5, Table 1) showing the hybrid (SM) and styrene (SS) diad. See the insert for a detailed view of the diad region.

ported a response factor (relative correction factor for use in eq. (1a) of 3 for the SM diad [14]. This response factor would yield a styrene NASL of 2.67, 1.96 and 1.41, respectively, for samples 1, 2 and 3. However, Wang's response factor seems to conflict with the NMR results obtained in the present study. The py-GC measurements of Wang et al. were performed using a different instrument, which renders the response factors not directly comparable. In this

work we measured the response factor of the SM diad by minimizing the error between the calculated styrene NASL from NMR results and the value calculated by GC using the `fminsearch` algorithm of MATLAB. The obtained response factor for the SM diad was 4.02, when using the total ion current, (TIC) and 5.48, when using the extracted ion currents (XIC) of m/z 91 and 145 of the SS and SM diads, respectively.

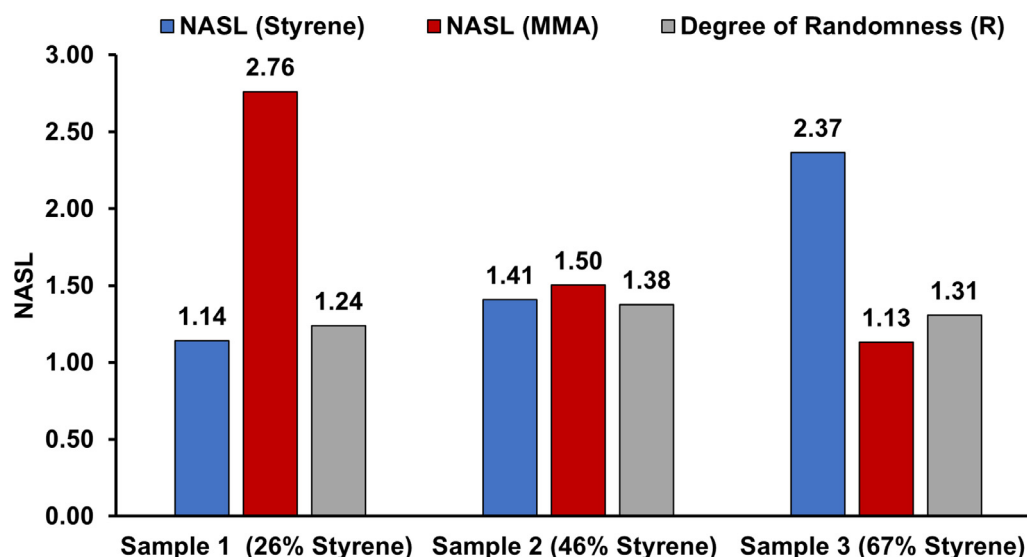


Fig. 6. Number-average sequence lengths (NASL, blue and red bars) and the degree of randomness (grey bars) for the three random copolymers (samples 1-3, Table 1) as determined by ^{13}C -NMR.

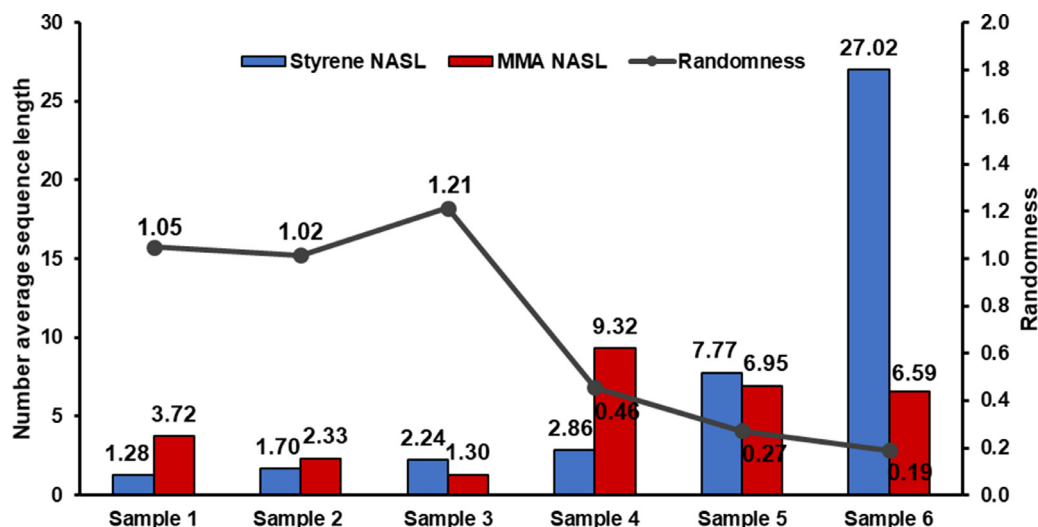


Fig. 7. Determined sequence parameters for the three random and three block samples (sample 1-6) calculated based on the XIC areas for SM and SS from the Py-GC-MS results using the NMR-derived correction factor of 5.48 for the SM dimer area.

It should be noted that sequence determination of these polymers by NMR is challenging, because the determined values are influenced by various factors, such as the tacticity. It is also demanding to obtain sufficiently high signal-to-noise values to reliably quantify the partially overlapping signals needed for sequence determination by ^{13}C -NMR [27]. Furthermore, the signals are convoluted to the extent that discrimination of various triads is challenging. Minimal spectral differences are observed between samples with differing compositions. Thus, the challenges in sequence determination by NMR are numerous, highlighting the importance of the present work as a practical and more straightforward approach from a data-analysis perspective, based on the excellent isomer resolution provided by GC Fig. 7. provides an overview of the characteristic sequence parameters for each sample after correction with the response factor based on the extracted-ion currents, which is deemed the most-sensitive method. The values obtained are in good agreement with those calculated based on the TIC.

The determined N_s values correlate with the determined values by NMR, although the absolute accuracy may be improved. The

N_m values, which are calculated by application of Eq. (4), seem to deviate more from the NMR data, which can be explained by small errors in the determined composition that heavily influence the determined N_m . This is a downside of the indirect determination of N_m . Furthermore, the response factor was optimized based on the N_s values, since those were directly determined by py-GC. Overall, the py-GC method also indicates a slightly alternating structure, but the determined randomness values are lower than indicated by the NMR measurements, especially for samples 1 and 2.

The sequence parameters obtained for the block co-polymers are clearly in line with expectations from synthesis. The obtained values for the degree of randomness clearly indicate a block-like structure. Furthermore, the determined N_s values increase with the block length, as expected. The sequence determination of block copolymers by NMR is very challenging, as it may not be possible to accurately detect the low-abundant hybrid triads. This highlights another favourable aspect of py-GC. The superior sensitivity allows the hybrid diad to be detected, even when its abundance is low, as is the case for block co-polymers.

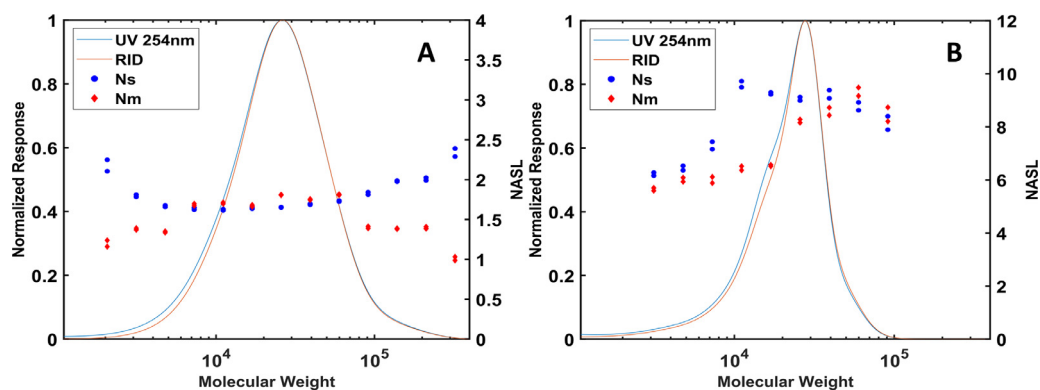


Fig. 8. Overlay of the normalized SEC UV and RID signals (left-side vertical axis) for a statistical copolymer (sample 2, Fig. 8A) and a block copolymer (sample 5, Fig. 8B) with the number average sequence lengths of styrene (blue dots) and MMA (red diamonds) indicated on the right-side axis. Note: the right-side Y-axis differs by a factor of 3 between Figs. 8A and B.

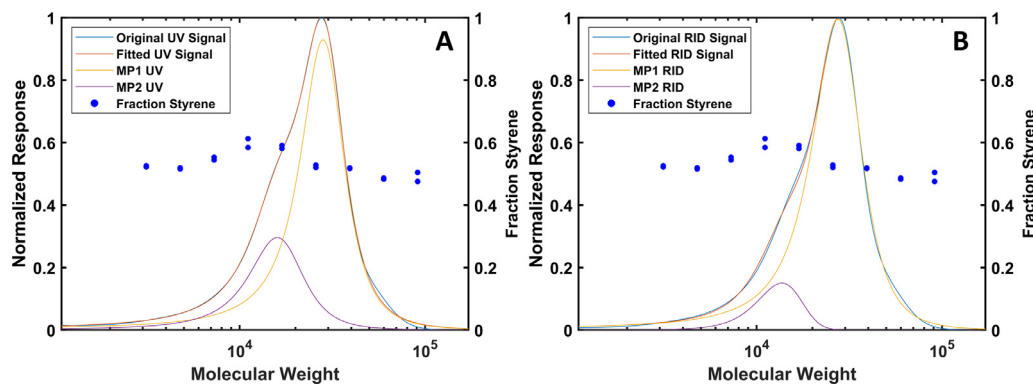


Fig. 9. Fitted modified-Pearson-VII distributions on both the UV (A) and RID (B) signal of sample 5 the normalized original signal (blue) is indicated on the left-side vertical axis. The reconstructed signal consisting of the two modified-Pearson fits (MP1 and MP2), is overlaid in orange. The weight fraction styrene determined by py-GC is indicated by the dots (right-side vertical axis).

3.3. Sequence determination across the MMD

To investigate whether SEC-Py-GC is suitable for determination of the sequence distribution as a function of molar mass, the SEC fractions (Section 2.2) were analyzed by Py-GC. The results can be seen in Fig. 8. The NASL of styrene (N_s) is seen to increase slightly for the low- and high-MM fractions (Fig. 8A). The low spread between the duplicate measurements even at lower concentrations suggests that the sequence is quantifiable in a repeatable manner (blue and red dots Fig. 8A). The NASL observed for the block co-polymer are significantly higher (Fig. 8B), as expected, and the values are less constant across the molar-mass distribution. A decrease in the NASL of styrene is observed at the low-MM end of the distribution, which can be explained by the decreased block length at lower molar masses.

To deduce the origin of the trend observed in the NASL of the block co-polymer (sample 5, Fig. 8B) across the MMD, deconvolution of the bimodal distribution was attempted. Two modified-Pearson-VII distributions were fitted to describe the full signal [43]. The results can be seen in Fig. 9.

The results in Fig. 9 suggest the presence of a secondary low-MM distribution that is more abundant in styrene. This distribution concurs with the increase in styrene sequence length observed in Fig. 8B. These observations are possibly due to polystyrene that is not attached to the PMMA block precursor in the second reaction step.

The results shown in Figs. 3 and 5-9 demonstrate that py-GC is an attractive alternative to NMR for co-polymer sequence determination. The advantages of py-GC over NMR are much greater

sensitivity (signal-to-noise ratios) and improved resolution between various subunits. While py-GC overcomes some of the limitations of other methods, it does have its own drawbacks, the main one being the lower quantitative accuracy of the determined sequence parameters, if not all relevant diads and/or triads are formed during pyrolysis. The online coupling of SEC to py-GC can offer a wealth of information on copolymer composition and sequence across the MMD. However, although such couplings have been demonstrated [35], the approach is still challenging and far from common. We have found the results obtained by py-GC-MS to be generally comparable to those obtained with NMR, although it is difficult to obtain reliable reference values from NMR. We have found the Py-GC-MS especially useful for the sequence determination of block copolymers, since the greater sensitivity allows quantification of the low-abundant hybrid dimer, which is difficult to observe with ¹³C-NMR. We aim to expand the application range of py-GC to other polymer systems, since the technique has so far seen limited application in polymer-sequence determination. The increased resolution might render py-GC applicable to ternary or even more complex co-polymer systems.

For the determination of the MMA block length accurate composition information as a function of MM is required. This information can be quite hard to obtain in practice, as shown in this work. In the low-MM region SEC-UV-RID tends to overestimate the styrene content, because of the higher number of end groups present. We have shown that py-GC can be used to reliably quantify the composition of block and random co-polymers using the devised mass-balance method with calibration.

4. Conclusion

The sequence parameters of various random and block copolymers was successfully determined using pyrolysis-GC and the application of the method to the offline monitoring of fractions collected from a liquid-chromatographic polymer separation was shown for the first time. An example was described, in which determination of chain-microstructure parameters was found to emphasize homopolymeric impurities in a block copolymer sample. Such impurities would be hard to detect with current methods and would require development of sophisticated HPLC methods. Although a fundamental challenge was encountered with the analyzed polymer system, poly(methyl methacrylate-co-styrene), the absence of MMA-rich diad and triad fragments, this could be overcome by fusion of data obtained by NMR and py-GC. Calibration with NMR enabled the determination of the number-average sequence lengths (NASL) and the degree of randomness (R) from py-GC data. Py-GC-MS was shown to be a powerful microstructure-sensitive detector for liquid chromatography of polymers where external calibration protocols are lacking. This technique revealed information that was not accessible with other size-exclusion-chromatography detectors.

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.

CRediT authorship contribution statement

Wouter C. Knol: Conceptualization, Methodology, Investigation, Visualization, Software, Formal analysis, Writing – original draft. **Till Gruending:** Supervision, Funding acquisition, Writing – review & editing. **Peter J. Schoenmakers:** Supervision, Funding acquisition, Writing – review & editing. **Bob W.J. Pirok:** Conceptualization, Supervision, Writing – review & editing. **Ron A.H. Peters:** Conceptualization, Supervision, Funding acquisition, Project administration, Writing – review & editing.

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