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Solvent suppression techniques for coupling of size exclusion chromatography and ^1H -NMR using benchtop spectrometers at 43 and 62 MHz

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Abstract

The characterisation of polymeric materials in their full complexity of chain length, monomeric composition, branching and functionalization is a tremendous challenge and is best tackled by tailored multi-dimensional coupled analytical and detection techniques. Herein, we focus on the improvement of an affordable but information rich 2D-method for polymer analysis: the online hyphenation of benchtop ^1H -NMR spectroscopy with size exclusion chromatography (SEC). The main benefit of this approach is correlated information of chain length (SEC) to chemical composition (^1H -NMR). Our setup combines SEC onflow with a benchtop NMR spectrometer at 43 or 62 MHz with chemical shift resolution as a robust detector. A detailed comparison of the two instruments is included considering, that only the 43 MHz instrument is equipped with a dedicated z-gradient enabling pulse sequences such as WET.

The main challenge of this method is the very low concentration of species of interest after chromatographic separation. At typical SEC conditions, the analyte dilution is typically more than a factor of 1000:1 in a protonated solvent. Therefore, an efficient solvent signal suppression is needed. In this article, several suppression pulse sequences are explored like WET, WEFT, JNR and a simple one-pulse approach – some for the first time on this hardware. By choosing an optimal method, signal strength ratios of solvent to analyte of 1:1 or better are achievable on flow.

To illustrate the broad range of possible applications, three typical cases of analyte to solvent signal proximity (no overlap, partial and full overlap) are discussed using typical polymers (PS, PMMA, PEMA) and solvents (chloroform and THF). For each case, several suppression methods are compared and evaluated using a set of numerical criteria (analyte signal suppression and broadening, solvent signal suppression, remaining solvent signal width).

Keywords

benchtop NMR, polymer analysis, chromatography, solvent suppression, combined method, SEC-NMR

Introduction

Today's functional polymers are everywhere: from displays to fuel cells, from medical implants to packaging or membranes for separation applications.[1] However, each polymeric material is in itself a complex mixture of chains with different length (molecular weight distribution, MWD), different monomers at different positions (copolymer, chemical composition distribution, CCD) and topology (such as linear, branched, star etc.). Additionally, often blends of different (homo-)polymers are used. In each of these “variables”, a polymeric material has a distribution of manifestations which are important for the material properties. Each dimension of the polymer needs to be investigated for a complete characterisation of the material.

The MWD of natural and synthetic polymers is, as a standard method, characterized by size exclusion chromatography (SEC). This is a solution based separation method where the underlying separation mechanism is based on an entropic mechanism utilizing the hydrodynamic radius of a dissolved molecule.[2-4] The experimental setup shares many similarities with liquid absorption chromatography (LAC). However, LAC separates according to molecular interactions with the column material and is mostly insensitive to molecular weight.

SEC alone gives information only on the MWD dimension of a polymer. As it separates due to solution size and the most used detectors in SEC, such as the differential refractive index (DRI) and viscosity detectors are only concentration sensitive or multi-angle laser light scattering (MALLS) detectors which are only molar mass sensitive.[2] Using these detectors, molecules with the same solution size but different chemical composition or topology are indistinguishable. Information on the other dimensions can be obtained using different methods such as other types of chromatography or spectroscopy. While this information can be gathered offline with dedicated methods, we propose the advantage of directly coupled methods which give a correlated picture e.g. the chemical composition at each molecular weight. Additionally, such methods can be used with more speed and routine once they are developed.

Herein, we focus on the online-coupling of SEC to ^1H -NMR spectroscopy. This method adds highly interesting information in the chemical composition dimension such as distribution of comonomers, functional groups or blended

polymers in the MWD. Due to these possibilities, the hyphenation of chromatography and ^1H -NMR has been widely explored for high-field NMR instruments in LAC.[5,6] Far fewer investigations have focused on the coupling with SEC (see below).[7-11]

The advantage of high-field NMR coupling is high resolution / selectivity towards chemical shift differences. However, the needed spectrometers are complex and costly especially compared to typical chromatography setups, consequently the usage has been greatly limited. Therefore, we focus on the usage of benchtop NMR instruments using permanent magnets up to 100 MHz (^1H) as detectors. These instruments have several advantages; (1) they are much more affordable and their footprint is in the range of a typical chromatography setup, (2) benchtop instruments are simple to maintain as they do not include liquid gas cooling. (3) The operation can be easily paused, allowing, with the small size, a simple transfer between applications or labs. Such instruments are robust in their operation which offers an upside in routine work such quality control or sample screening.

The typical challenges faced by SEC-NMR (and HPLC-NMR) of polymers are on-flow measurements, very low analyte concentrations ($\ll 1$ g/L), protonated solvents with strong and possibly overlapping resonances of interest. So far, only the usage of highly abundant ^1H and ^{19}F nuclei in NMR spectroscopy have been used in coupling methods due to the low final concentration. The relevant differences of SEC to HPLC for NMR applications include the absence of solvent gradients. Moreover, the large polymer chains exhibit significant NMR relaxation time differences (T_1 and T_2) compared to the solvent molecules and show fairly broad NMR signals.

The listed challenges warrant a strong focus for any SEC-NMR investigation on signal-to-noise ratio (SNR) optimization, usage of solvent signal suppression schemes and keeping flow effects within limits (in-flow and out-flow effects, for explanation see e.g. Gouilleux et al.[12]) This is true for all hyphenation techniques but the hard- and software solutions are highly dependent on the specific application. The focus in this publication is on the optimum approach for coupling SEC to a medium-resolution (MR) / benchtop NMR, which is somewhat different from the one for e.g. high field NMR couplings where pulsed gradients are easier to realise.

First investigations of SEC-NMR high-field couplings were undertaken by Hatada et al. and were later extended by Albrecht et al and Hiller with Pasch et al.[7-11] A first attempt of SEC-NMR coupling with a benchtop spectrometer was attempted by Cudaj et al. using a 20 MHz spectrometer.[13,14] While giving some promising first results, limits in sensitivity made experiments with typical SEC concentrations difficult and chemical resolution was too limited for many applications. However, development of benchtop NMR has been swift in recent years towards better hardware (e.g. higher fields and resolution) and pulse sequences (possible due to field homogeneity).[15,16] These developments brought experiments at typical SEC concentrations to our focus. We already reported the proof of concept for the combination of a SEC system with a state-of-the-art medium resolution ^1H -NMR spectrometer at 62 MHz as a chemical sensitive detector.[17] In a following article, we reported an optimisation of SNR and SEC systems and methods to achieve highest sensitivity.[18] Work by Sabatino et al. followed a similar approach.[19] In the present article, we focus on NMR pulse sequences used in the coupling for best solvent signal suppression while retaining analyte signals.

In this work, several solvent signal suppression pulse sequences are evaluated on a 62 MHz spectrometer with only shim gradients and compared to a 43 MHz instrument featuring a (z) gradient probe, which has not been available for the 62 MHz NMR instrument so far. The general setup is depicted in **Figure 1** and is the same for the 43 MHz and the 62 MHz instruments.

Figure 1: General setup for SEC-NMR measurements featuring an isocratic pump with an external degasser unit, a manual injector, a semi-preparative SEC column, the medium resolution (MR)/benchtop NMR instrument including the flow

cell and a downstream differential refractive index detector (DRI). Reproduced from Ref. [16] with permission from the Royal Society of Chemistry.

Particularly for ^1H -NMR spectroscopy in protonated solvents at medium field, a sufficient solvent signal suppression is indispensable. The methods used can be divided into two categories: (1) solvent signal suppression prior to data acquisition to make optimal use of the available analogue to digital converter (ADC). This is done by specially designed pulse sequences. (2) post acquisition data treatment by numerical solvent signal subtraction in the data. The use of solvent suppression pulse sequences will be discussed first.

It is crucial to suppress the solvent signals to some extent prior to acquisition due to several reasons.[20] A non-suppressed solvent resonance is often more than 1000 times more intense than the resonances of interest which leads to poor utilization of the receiver electronics and the ADC limiting the relative sensitivity. Moreover, effects like radiation damping or the so-called faraway water effect cause peak broadening which shrinks the quantifiability of signals near the solvent resonance.[21]

Suppression techniques can be grouped by their working principle. The first group of methods relies on selective excitation and saturation of the solvent signal prior to acquisition by methods such as presaturation or **Water suppression Enhanced through T_1 effects (WET)**. The second group of pulse sequences uses frequency specific editing of the signals of interest by removing certain frequencies by, e.g. Jump-and-Return pulse sequences or **WATER** suppression by **GrAdient Tailored Excitation (WATERGATE)** or excitation sculpting. The third group considered is the exploitation of differences in T_1 relaxation between solvent and analyte such as 90-spoil (see below), progressive saturation or inversion recovery / **Water Eliminated Fourier Transform (WEFT)**.

Probably the most straightforward approach to solvent suppression in static measurements is the presaturation technique by simply applying a long low-power selective pulse on the solvent resonance.[22] For on-flow measurements, this approach is much less suitable as long repetition times are needed, limiting obtainable SNR and a strong apparent relaxation due to inflow of new solvent is observed, strongly limiting the otherwise efficient suppression. Moreover, suppressing multiple solvent signals, e.g. the two THF multiplets introduces further challenges.

A potentially faster way to saturate the respective solvent resonance is the WET-approach:[23] This pulse sequence is generally made up of four shaped pulses selective on the solvent resonance yielding an effective 90° pulse and is compensated for inaccuracies in pulse widths and mistuned probes. The use of pulsed field gradients complementing every single WET-subunit is capable of dispersing the longitudinal solvent magnetization and allows a high degree of solvent suppression even under flow conditions.[12,24]

Shorter repetition times are possible with frequency selective pulse sequences of the **J**ump-a**Nd**-**R**eturn (JNR) type.[25] These are combinations of constantly spaced pulses with their duration following the binomial coefficients. The simplest version referred to as Jump and Return 11 is a sequence $90^\circ_x - \tau - 90^\circ_{-x}$. With the solvent magnetization on-resonance, no rotation occurs during the delay and there is practically no excitation for this frequency after the return-pulse. All other nuclei maintain at least some transversal magnetization and can be detected. These methods allow an intense suppression of analyte signals but, depending on the chosen interpulse delay, only parts of the whole spectrum can be displayed and phased correctly.

Another frequency selective method is the so-called excitation sculpting or its enhancement the WATERGATE sequence.[26] This sequence is a quite popular approach and its simplest form is made up of a spin-echo sequence with two symmetrical gradients. An undesirable side-effect is the so-called *J*-modulation around the suppressed solvent resonance, which potentially prohibits quantitative peak analysis near the solvent signal. This was the main reason for not pursuing this approach in this work. Nevertheless, WATERGATE has been applied to coupled LAC-NMR systems at high fields.[6]

A very appealing circumstance in NMR spectroscopy of macromolecules is the significant difference in the longitudinal relaxation time T_1 between polymers (200–500 ms at 62 MHz) and the small solvent molecules (typically 1–2 s at 62 MHz under SEC-conditions) as the solvent is degassed and triplet oxygen content substantially reduced. This fact can also be exploited for the use of solvent signal suppression. The simplest way to do so is keeping the repetition times very short, allowing the polymer FID to decay completely whereas the residual solvent FID is destroyed by a spoil gradient after acquisition before the next transient begins. This was implemented in the 90-spoil pulse sequence described in detail later.

The WEFT sequence, is a method for solvent suppression in static samples exploiting the zero-intersection of the longitudinal solvent magnetization similar to an inversion recovery experiment.[27] Theoretically, the experiment would have to be conducted in a way that an effective 180° pulse is followed by a waiting time corresponding to the effective T_1 relaxation time of the solvent molecules to achieve a fully suppressed solvent signal when exciting the system with a 90° hard pulse. However, it is possible to achieve almost full solvent suppression with shorter repetition times, since the apparent T_1 of the solvent resonance decreases with decreasing recycle delay. Exploiting this fact makes WEFT a very interesting approach for on-flow solvent suppression. To the best of our knowledge, this pulse sequence has not been reported for measurements under flow conditions, yet even though the so-called in-flow effect causes faster T_1 relaxation due to the shortened residence time.[28] Both T_1 selective approaches are not expected to yield a high degree of analyte signal suppression.

In addition to these pulse sequences, the acquired raw data can be afterwards subjected to mathematical post-processing (see Höpfner et al and Botha et al. [17,18]). The nature of SEC-measurements allows the acquisition of pure solvent spectra in the first few minutes of every run. For constant solvent signals, a numerical subtraction of the full solvent signal should be straight forward. However, due to variations in the chemical environment of the protons in the presence of analyte molecules, slight changes in the peak shape prevent effective subtraction in this area of the elugram. Additionally, long- and short-term fluctuations of the strong solvent signal can cause additional difficulties. It was found, that a combination of both concepts, pulse sequence and numerical subtraction, is most efficient as discussed in the main part.

This work is focused on finding the best pulse sequences for this coupled method. These pulse sequences should be able to gather as much signal to noise per time as possible and reduce unwanted solvent signals efficiently. Both must be possible in an onflow condition with a high repetition rate. The high repetition rate is used to maximize the sensitivity but sacrifices the quantifiability of the recorded spectra. This would have required the use of a significantly longer repetition time, typically 5 times the longest T_1 . [29] Special emphasis is given to compare several pulse sequences that need a designated z-gradient (WET-type) to those which don't need this gradient for typical polymer samples (PS, PMMA,

PEMA) in SEC. Consequently, two spectrometers are used: a 43 MHz with a designated z-gradient and a 62 MHz without a z-gradient but with better sensitivity and resolution. An extensive set of criteria is presented to evaluate the pulse sequence performance in typical analytical problems of the method.

Materials and Methods

Used materials

As eluents tetrahydrofuran (THF, SEC-grade, stabilized with butylhydroxytoluol (BHT), Scharlau, Hamburg, Germany) and chloroform (SEC-grade, stabilized with 2-methyl-2-butene, VWR, Bruchsal, Germany) were used as received. The polymers were narrow dispersity reference materials and obtained from different sources as given in **Table 1**. Static NMR spectra of the polymers are shown in the supporting information **Figure SI1**.

Table 1: Designations, sources and characteristics of the polymer samples used in this study.

Designation	Polymer	Sources	M _w / kg/mol	Dispersity Đ / ---
PS30k	Polystyrene (PS)	Polymer Labs	30.9	1.02
PS90k	PS	In-house synthesis	90.0	1.10
PMMA30k	Polymethyl methacrylate (PMMA)	In-house synthesis	31.0	1.08
PEMA78k	Polyethyl methacrylate (PEMA)	In-house synthesis	78.0	1.12

NMR-Spectrometer

The NMR and SEC-NMR measurements were carried out on two different benchtop NMR spectrometers from the Spinsolve series (Magritek, Aachen, Germany). A Spinsolve 60 with a ¹H Larmor frequency of 62 MHz was equipped with an optimized single channel ¹H probe head but no gradient unit. A Spinsolve 40 with a ¹H carrier frequency of 43 MHz was also equipped with a single channel probe head but had a dedicated z-gradient with a maximal strength of

approximately 160 mT/m. Both instruments carry a permanent magnet in form of a Hallbach array with a bore that allows the usage of standard 5 mm outer diameter (O.D.) tubes or flow cells. Both feature a 15-channel electrical shim system using shim terms up to the 3rd order. The pulse sequences are freely programmable using the PROSPA language. Data acquisition on the Spinsolve 40 is performed using the PROSPA V354 software while on the newer Spinsolve 60 the Spinsolve Expert V1.21 software is available and was used. The pulse programs cannot be exchanged directly between the two software systems as some commands are inconsistent and the instructions need to be recompiled. A cross-compiler function is available.

SEC-Setup

In connection with both NMR spectrometers, an Agilent 1260 Infinity SEC system (supplied by PSS, Mainz, Germany) is used for SEC measurements. It consists of an in-line degasser, isocratic pump, manual injector (Rheodyne 7725i, 500 μ L sample loop, 6 ports) and differential refractive index (DRI) detector. The chromatographic separations were performed with PSS SDV linear M semi-preparative columns (300 x 20 mm I.D., 10 μ m particle size, mixed bed), one for THF and one for chloroform. One NMR spectrometer at a time was coupled as an additional detector to the SEC system between the column and the DRI detector using a custom-made flow cell (see Figure 1). The flow cell has an active region in the area of the NMR coil with 3.4 mm I.D. over 15 mm length. The flow cell has been described in more detail elsewhere.[18] The flow rate is 1.0 mL/min, leading to a run time of 85 min for one experiment. The sample concentrations were 2 and 4 g/L for Spinsolve 60 and 40, respectively to account for differences in sensitivity as discussed in the evaluation. This corresponds to an injected mass of either 1 or 2 mg (0.5 mL injected volume). A column loading of 2 mg is already close to the overloading limit of the semi-preparative column (depending on the exact conditions).[18]

SEC-NMR measurements

Before the combined measurements, the magnet was shimmed on the flowing eluent giving a typical line width for chloroform of 0.7 Hz and 0.4 Hz for Spinsolve 60 and 40, respectively. The transmitter frequency is set to the highest solvent peak and the receiver phase is optimized manually. The values are kept constant over the course of one SEC-NMR run. The NMR data is acquired with one of the pulse sequences described below. The receiver gain is optimized for each

combination of pulse sequence and instrument (62 or 43 MHz) separately. A non-interactive program collects and co-adds 4 transients into one FID and continuously records and saves FIDs over the course of the SEC run without any interdelay. Over the length of one SEC experiment between 2600 and 1300 FIDs are collected depending on the length of the pulse sequence (see below). The NMR spectra are, therefore, independent measurements, but give a 2D data set where chemical shift/FID time is one dimension and eluent time the second. The full 2D data set is collected during the experiment and evaluated later as described below.

NMR pulse sequences

Several different pulse sequences were used to test the solvent suppression efficiency of each sequence. The sequences were built by combining different preparation, excitation and read-out blocks as shown in **Figure 2** to obtain the tested sequences: 90-spoil, JNR1331, WEFT, WEFT-spoil, WET-90, WET-CP and WET-180-NOESY. All pulse sequences were used inside a similar master program that recorded continuously as described above. The 90° hard pulse length was determined to be 12 μ s and 6.35 μ s for Spinsolve 60 and 40, respectively, at 0 dB pulse damping (full power, 1 mW). The 180° hard pulses were double the length of the 90° pulse.

Figure 2: The building blocks that were combined in this work to obtain solvent suppression pulse sequences can be grouped in the categories of preparation, excitation and read-out blocks where (d) combines features of the former two). The blocks are named as follows: (a) WEFT-block, (b) WET-block, (c) WET-180-block, (d) JNR1331-block, (e) 90°-pulse read block, (f) composite pulse read block, (g) 1-D-NOESY-block, (h) FID-block and (i) FID-spoil-block. For details on the blocks see text.

The preparation blocks are the WEFT block, see Figure 2 (a), consisting of a 180° hard pulse followed by an optimized waiting time τ_{WEFT} . The optimization procedure for the parameter τ_{WEFT} is given in the main text, it is in the order of 500 ms within this work. The WET block, Fig. 2 (b), consists of four Gaussian shaped soft pulses with a power of -31.8 dB (1011 Hz), -31.5 dB (1047 Hz), -33.5 dB (832 Hz) and -30.4 dB (1189 Hz) and a duration of 82 ms for each as given in previous work for this instrument.[12] The frequency of the soft pulses was slightly offset from the solvent peak by -8.5 Hz and -10 Hz for chloroform and THF, respectively (see as well application case 3 for the optimization of the offset). The z-gradient pulses followed each soft pulse with a duration of 2 ms and halving the power for each consecutive gradient pulse starting from 85 % of maximum gradient strength. The WET-180 block, Fig. 2 (c), is similar to the WET block but after the last soft pulse a 180° hard pulse is added, and the strength of the last soft pulse is reduced to -39.6 dB (412 Hz).

The excitation block can be a simple 90° hard pulse, see Figure 2 (e). The second option is a composite 90° pulse consisting of four 90° pulses with an interpulse spacing of 5 μs , see Figure 2 (f). The last option is a 1D-NOESY block: two 90° hard pulses (spacing 10 μs) are followed by the mixing delay adjusted in this work to 6 ms and a final 90° hard pulse, see Figure 2 (g).

In the JNR1331 pulse sequence, see Figure 2 (d), the preparation and excitation part are combined by a train of four hard pulses with their respective length given by the binomial sequence 1a, 3a, 3a, 1a where 8a is the duration of one 90° pulse or 12 μs at 62 MHz. All interpulse delays τ_{JNR} are the same and adjusted to focus the area of interest in the spectrum.[25] In this work, focusing on the aliphatic protons in chloroform a delay of 2 ms is used.

Read-out blocks were either a simple FID with 100 μs acquisition delay, see Figure 2 (h), 2k data points and 200 μs dwell time giving a length of 410 ms. For the weaker suppression techniques, a 90-spoil block, Fig. 2 (i), was used as the FID had not decayed to noise level during the acquisition period. For the spoil block, after the FID as described above, a 5 ms waiting time was added followed by a crusher gradient for 20 ms using the linear terms of the shim coils and further 5 ms of waiting time. The strength of the crusher gradient was 5 000 a.u. which roughly corresponds to 0.5 mT/m.

Numerical data processing

The 2D SEC-NMR spectra evaluation was automated using a self-written MATLAB script. The principle evaluation procedure has been disclosed in previous publications,[17,18] but some specific procedure was found necessary for data produced by the new pulse sequences employed in this work. The 2D raw data is in the form of FIDs which are in the first step apodized (Gaussian lb 1.8 Hz) and zero filled to 16k. After Fourier transform, the spectra are corrected in a second step for 0th and 1st order phase offsets. For the pulse sequences with a strong, undistorted solvent signal remaining (90-spoil) the 0th order phase was corrected by minimizing the sum of disperse signal of the solvent peak(s). In the other cases, a constant 0th order phase was used. In both cases, a constant 1st order phase correction was used, which was mostly dependent on the solvent used. The next step depends on the pulse sequence.

If the pulse sequence produced a (residual) solvent signal of stable intensity the data was smoothed along the elution dimension as a third step and a reference spectrum is subtracted as the forth step. This was used for all pulse sequences except WEFT-90-spoil and JNR1331. For JNR and WEFT-based sequences the third step was subtraction of a reference spectrum (with variable amplitude) and the forth step was smoothing along the SEC elution dimension. This order of steps was found to be beneficial due to strong fluctuations in solvent intensity but not spectral composition of the polymer.

The smoothing along the SEC dimension is done by applying a Gaussian filter with a bandwidth (sigma) of 32 s at each frequency / spectral point. For the reference subtraction, a reference is computed by averaging analyte free spectra in the beginning of each run. The reference is subtracted from each spectrum with an adjusted intensity. The solvent intensity of the reference spectrum is adjusted to the highest solvent signal in the current work spectrum.

As the fifth step, the analyte peaks are automatically detected using a threshold method. The sixth step is a baseline correction along the elution (SEC) dimension performed by adjusting a 2nd order polynomial to the analyte peak free region for each frequency / spectral point. This corrects for slow drifts in the residual solvent signals and other constant signals. Finally, statistics for the solvent and analyte, are calculated as detailed below and the data is visualized.

Results and discussion

Evaluation criteria for solvent suppression techniques

The theoretically best pulse sequence would eliminate all solvent signals completely while not hampering the sensitivity and selectivity of the analyte's signals of interest in all parts of the spectrum. While a "perfect" method is not known and probably does not exist, it is worthwhile to discuss criteria to benchmark the existing methods against these ideals not only in a qualitative way but also by a quantitative approach. The criteria proposed are solvent signal reduction (desirable), analyte signal reduction (undesirable), analyte signal broadening (undesirable) and available baseline (desirable) as introduced herein. The available baseline is a concept inverse to solvent signal width but a better handled concept in our case as discussed below. These criteria are in general universal, but to be quantified they need to be assigned and measured for every given case of solvent, analyte and setup and more importantly the weighting is largely dependent on the analytical problem at hand. Here, we focus on the application to the hyphenation of medium resolution NMR to SEC and will describe where we see the optimum in this case.

The first desired criterion is a low intensity solvent signal to prevent receiver overloading and other detrimental effects discussed above. However, beyond a certain threshold, e.g. same/lower intensity as analytes' signals, a further reduction of solvent signals are not always needed. This goal can be quantified directly by the solvent signal-to-noise ratio (SNR_S) given for the major solvent peak. Here, two values are given for each dataset: the raw SNR_S is the value before numerical solvent suppression (but including suppression by the pulse sequence) and the final SNR_S is the value including numerical suppression. To compare different methods, the suppression ratio SR_S is defined as the ratio of unperturbed solvent signal intensity divided by suppressed intensity. The unperturbed intensity is taken from a reference dataset with the same repetition time and injected mass using the 90-spoil sequence. The SR_S value should be as high as possible.

As the counterpart to the first criterion, the analytes' signals should be suppressed as little as possible. This is a very important point in our application as the signals are already weak. For quantification of the suppression of analyte signals, the final signal-to-noise ratio of the analyte (SNR_A) is given for the peak(s) of interest. This value should be as high as possible.

The selectivity in the NMR dimension is benchmarked by the analyte's peak width. The analyte's peak width is quantified by the full width at half height of an analyte ($FWHM(A)$) peak well separated from the solvent signals. In this publication, we are only concerned with the signal width in the spectroscopic dimension, for an in-depth discussion for the width in the chromatographic dimension see Botha et al.[18]

As a counterpart, the peak width of the solvent is monitored as well and is to be optimized to be as low as possible. Typically, solvent peak FWHM decreases when the intensity is suppressed. However, it was found that many effective suppression methods such as WET based approaches distort the solvent signal strongly, resulting in a multitude of irregular peaks. Determination of FWHM on these residual (multiplet) signals is ill defined and very error prone, consequently a different approach was utilized. As basic idea, a good solvent suppression retains as much as possible of the baseline without solvent artefacts and fluctuation. This region where analyte peaks can clearly be identified and quantified as they develop and vanish over time is called available baseline here. A method is needed to quantify how much of the baseline is obscured by solvent fluctuations too high to analyse the analyte peaks there.

Therefore, we propose a fluctuation based criterion relying on the 2D nature of the obtained data (**Figure 3**). First, the standard deviation (SD) of each NMR elugram, i.e. all 1-D cuts along time at one NMR resonance frequency of the 2D data set, is calculated as shown on the top of Figure 3. Then, the SD values are plotted as a function of chemical shift (other 1-D dimension). This yields a spectrum-like plot, where the solvent peaks and constant background are displayed as their fluctuation over time (bottom of Figure 3). The regions with high fluctuations are improper for any quantitative analysis. Consequently, a high threshold of 5 times the SD in the signal free region is defined and only the parts where the SD is higher are defined as unusable, denoted by the remaining (solvent peak) width, W_{rem} . This value should be as low as possible. The threshold value is arbitrary but was found to work reliably for the given problem. If an analyte peak is close to the solvent peak it can obscure this evaluation. In this case, W_{rem} is calculated only from the solvent peak centre to the far side of the peak and doubled (for $CHCl_3$ as solvent) or calculated on the other solvent peak (for THF as a solvent).

Figure 3: Schematic of the evaluation of the unobstructed baseline by the solvent signal here at 7.25 ppm. The parameter W_{rem} is calculated from the area in the lower graph where the standard deviation is above the threshold (red lines). For further explanation see the text.

These four criteria have been evaluated automatically for all pulse sequences and samples to guarantee reliability. In this method, a strong importance is given to the strength of analyte signals (SNR_A) as their intensities due to dilution are already low. The SR_S is somewhat less important as will become clear in the forthcoming results. W_{rem} is important for some possible applications and also decisive in identifying the best method.

Comparison of the performance of 43 and 62 MHz spectrometers

In the scope of this work, two different spectrometers were used as explained in the introduction due to their individual benefits for using solvent suppression pulses sequences. The 62 MHz spectrometer provided better spectral resolution while the 43 MHz spectrometer offers more flexibility in terms of pulse sequences (e.g. WET) due to a dedicated z-gradient. In this section, the results of the two spectrometers are compared by measuring SEC-NMR on PMMA30k in CHCl_3 using the simple 90-spoil sequence. The results are shown in **Figure 4** and the results for the numerical criteria are given in **Table 2**.

Figure 4: SEC-NMR results of PMMA30k in CHCl_3 on the 62 MHz (left) and 43 MHz (right) spectrometers recorded by using the 90-spoil sequence (see Figure 2 (e+i)). On top, contour plots of the full region of polymer elution are given with 15 evenly spaced intensity lines (from 0 to maximum analyte peak height) and below the spectra extracted at the highest points of the polymer signals are shown (highest analyte peak is normalized to 1). The same scaling is applied to all subsequent plots as well. For static spectra of the compounds under investigation see the supporting information.

Since the two spectrometers feature different magnet setups, field strengths and electronics, it was necessary to evaluate the reachable SNR first. To counter the

expected drop in SNR, the injected mass was doubled to 2 mg PMMA for the Spinsolve 40. Several factors influence the reachable SNR. According to a simple approximation, SNR increases with an exponent of 1.5 with respect to the B_0 field for a constant number of scans. The SNR for the Spinsolve 60 should consequently be higher by $(62/43)^{1.5} = 1.73$. Additionally, it was not possible to reach the same repetition time on the Spinsolve 40 due to slower spectrometer or PC hardware which was expected to result in a SNR by factor $(500/650)^{0.5} = 0.88$ worse for the Spinsolve 40.[30] The doubling of injected mass for the Spinsolve 40 instrument will also double SNR as shown in previous work.[17] Therefore, the expected SNR (at higher injected mass) should be approximately similar for the Spinsolve 40 and for the Spinsolve 60 in the scope of the experiments for the higher loading as described.

Table 2: Evaluation criteria for solvent suppression in SEC-NMR of PMMA30k.

Spectrometer (^1H)	62 MHz	43 MHz
Inj. mass polymer [mg]	1.00	2.00
Repetition time [s]	0.50	0.65
$SNR_A(-\text{O}-\text{CH}_3)$	127	150
$FWHM_A(-\text{O}-\text{CH}_3)$, [Hz]	6.42	6.23
$SR_S(\text{CHCl}_3)$, suppression ratio of solvent	194	62.0
(Raw and) Final $SNR_S(\text{CHCl}_3)$	(32 800) 169	(35 500) 570
$W_{rem}(\text{CHCl}_3)$, width of distorted baseline [ppm]	0.26	0.60

Comparing the two spectrometers, the SNR_A for the Spinsolve 40 is 16 % higher than expected. This might be a feature of the different magnet design or material or achievable receiver gain. The NMR peak width is similar, the separation on the baseline is improved for 62 MHz NMR as is easily visible in Figure 4. The suppression is better on the Spinsolve 60 in both the residual solvent strength and the recovered baseline. These values serve as a reference to benchmark against all further pulse sequences and solvent / analyte combinations discussed in the next sections.

Application case 1: Measurements of PMMA in chloroform

The first and easiest case to be discussed is a full baseline separation between the solvent and analyte's signals. Typical examples would be the spectra of PMMA in chloroform where all resonances are well separated or the aliphatic resonances of PS in chloroform and the aromatic resonances of PS when recorded in THF. We consider the first case in more detail, while some aspects of the other cases will be included at the end of this section.

Results for SEC-NMR data of PMMA30k in chloroform measured on Spinsolve 40 are shown in **Figure 5** for the pulse sequences 90-spoil (same dataset as in previous section) and WET-CP. The results of the numerical evaluation are given in **Table 3**.

Figure 5: SEC-NMR results of PMMA30k in CHCl_3 recorded on the 43 MHz spectrometer using the 90-spoil sequence (left, see Figure 2 (e+i)) and WET-CP (right, see Figure 2 (b+f+h)).

Table 3: Evaluation criteria for solvent suppression in SEC-NMR of PMMA30k in CHCl_3 (well separated case) comparing two pulse sequences for solvent suppression used on the 43 MHz spectrometer.

Pulse sequence	90-spoil @ 43 MHz	WET-CP @ 43 MHz
Inj. mass polymer [mg]	2.00	2.00
Repetition time [s]	0.65	1.00
$\text{SNR}_A(-\text{O}-\text{CH}_3)$	150	121
$\text{FWHM}_A(-\text{O}-\text{CH}_3)$, [Hz]	6.23	6.54
$\text{SR}_S(\text{CHCl}_3)$, suppression ratio of solvent	62.0	987
(Raw and) Final $\text{SNR}_S(\text{CHCl}_3)$	(35 500) 570	(55.0) 29.0
$W_{\text{rem}}(\text{CHCl}_3)$, width of distorted baseline [ppm]	0.60	0.19

When comparing SNR values for the two data sets, it has to be noted that the repetition time for the WET-CP pulse sequence is longer (1 000 ms) than for the 90-spoil sequence (650 ms) due to the duration of the four (long) soft pulses. The obtainable SNR_A per unit time is predicted to be lower for WET-CP by a factor of $(1000/650)^{0.5} = 1.24$. This is in very close agreement to the measured SNR values which show a reduction of 25 % for WET-CP. The solvent suppression is already very good for the WET-CP sequence before numerical subtraction (SNR of solvent signal is half of the highest analyte peak) and consequently only improved by a smaller amount (factor 2) by the numerical methods. The width of the distorted baseline W_{rem} is only one third for WET-CP when compared to the 90-spoil sequence. The influence on the initial solvent peak width is negligible. In conclusion, WET-CP offers a slightly reduced sensitivity but a great increase in solvent suppression capability.

Application case 2: Measurements of PS in chloroform

The second case of interest is partial overlap between the solvent resonances and the signals of the analyte. For polystyrene in chloroform, the aromatic signals are two broad multiplets with the upfield one centered at 7.0 ppm close to the chloroform resonance at 7.26 ppm which has a width at 0.55 % of ca. 15 Hz at 62

MHz and onflow conditions. For polystyrene in chloroform, the aliphatic signals (0.9 – 2.1 ppm) are baseline separated from the solvent and can be treated in a similar fashion to the PMMA signals described in the section above. Here, first pulse sequences without gradient (62 MHz spectrometer) are presented: Jump and Return and 90-spoil and then those with gradient (43 MHz spectrometer) WET type sequences.

The results of the Jump-and-Return pulse sequence, more precisely the 1331 version,[25] are given in **Figure 6** and compared to the results of 90-spoil sequence.

Figure 6: SEC-NMR results of PS30k in CHCl_3 recorded on the 62 MHz spectrometer using the 90-spoil sequence (left, see Figure 2 (e+i)) and Jump-and-Return 1331 (right, see Figure 2 (d+h)). Please note that due to a change of the column, the same analyte does not elute at exactly the same volume in both measurements.

The JNR1331 measurement is carried out with an optimized interpulse delay $\tau_{\text{JNR}} = 2$ ms to display the aliphatic protons of the PS sample with the carrier frequency set to the chloroform resonance. It must be noted that the aromatic PS signals close to the CHCl_3 resonance are not visible at all in the resulting spectra due to the nature of the pulse sequence, a clear disadvantage. A repetition time of 500 ms was possible for the 90-spoil and JNR1331 sequences. However, the

given numbers for SNR (**Table 4**) are given for different analyte concentrations. The injected mass was doubled for JNR1331 to obtain a higher (doubled) SNR.

Table 4: Evaluation criteria for solvent suppression in SEC-NMR of PS30k in CHCl_3 (well separated case for aliphatic PS protons) comparing 90-spoil and JNR1331 at 62 MHz and 0.5 s repetition time.

Pulse sequence	90-spoil @ 62 MHz	JNR1331 @ 62 MHz
Inj. mass polymer [mg]	1.00	2.00
$\text{SNR}_A(\text{PS aliphatic})$	39.0	23.0
$\text{SR}_S(\text{CHCl}_3)$, suppression ratio of solvent	142	549
(Raw and) Final $\text{SNR}_S(\text{CHCl}_3)$	(36800) 260	(67) 67
$W_{\text{rem}}(\text{CHCl}_3)$, width of distorted baseline [ppm]	0.24	0.31

A suppression of the chloroform resonance by a factor of 490 was possible considering the pure pulse sequence and the resulting solvent intensity is on the same order of magnitude as the ^{13}C -satellites. The eluent spectra in JNR1331 exhibit a very narrow residual linewidth and gave the impression that this method yields a very good peak base width. Objectively considered, this criterion does not prove useful here because signals in parts of the spectrum, other than the adjusted optimized positions, are out of phase and not usable. In combination with a suppression of the analyte signals by SR_A of maximum 0.29 for the well displayed signals, the JNR1331 approach does not fulfil the requirements of SEC-NMR.

Increasing the interpulse delay allows signals closer to the suppressed resonance to be displayed.[25] In the case of PS, $\tau_{\text{JNR}} = 30$ ms allows displaying the aromatic PS signals whereas the aliphatic signals are not visible any more. However, the SR_A in this case is around 20 and the repetition time increases strongly in these experiments (measurements not shown here). The long repetition time also decreases the time normalized suppression efficiency for the solvent, which results in a SR_S of roughly 10. Overall, the performance of the JNR1331 pulse sequence requires a lot of knowledge about the sample to allow parameter-optimization in advance and is not a viable option for this application.

The resonances of the aromatic PS protons in chloroform are an excellent example to investigate the case of analyte signals partially overlapping with the feet of the unperturbed solvent resonance.

Figure 7: SEC-NMR results of PS30k in CHCl_3 recorded on the 43 MHz spectrometer using the 90-spoil (top left, see Figure 2 (e+i)), WET-90 (top right, Fig. 2 (b+e+h)), WET-CP (bottom left, Fig. 2 (b+f+h)) and the WET-180-NOESY pulse sequence (bottom right, Fig. 2 (c+g+h)).

Figure 7 features SEC-NMR results of PS30k in chloroform for 90-spoil (same data set as above) at 62 MHz, WET-CP, WET-90 and WET-180-NOESY at 43 MHz using a pulsed z-gradient for the WET derivatives. For comparison of SNR values, all SEC-NMR runs, in Figure 7, were performed using a repetition time of 1 s and 2 mg injected sample mass. The corresponding numerical results are given in **Table 5**. From the solvent signal suppression point of view of the raw data, the

WET-CP approach apparently is the most powerful method suppressing the solvent intensity by a factor of 590 without affecting the analyte resonances significantly more than the other WET sequences. However, even though the raw SNR_S of WET-180-NOESY is an order of magnitude higher than for the other WET sequences, the numerical data treatment yields a solvent SNR_S , which is even better. This is a result of stable remaining solvent signal.

Table 5: Evaluation criteria for solvent suppression in SEC-NMR of PS30k in $CHCl_3$ (partially overlapping case) at 43 MHz, comparing the WET-90, WET-CP, WET-180-NOESY and the 90-spoil pulse sequence (repetition time 1 s for all). SNR values for the solvent denoted by * are only shoulders on the analyte signals and therefore difficult to quantify.

Pulse sequence	90-spoil @ 43 MHz	WET-90 @ 43 MHz	WET-CP @ 43 MHz	WET-180-NOESY @ 43 MHz
Inj. mass polymer [mg]	2.00	2.00	2.00	2.00
SNR_A (PS aliphatic)	42.0	36.0	35.0	23.0
$SR_S(CHCl_3)$, suppression ratio of solvent	43.0	592	327	1070
(Raw and) Final $SNR_S(CHCl_3)$	(32 000) 736	(309) 54.0*	(54.0*) 98.0*	(2150) 30.0*
$W_{rem}(CHCl_3)$, width of distorted baseline [ppm]	0.26	0.39	0.42	0.22

Moreover, in this case, other evaluation criteria like the residual solvent peak base width, gain further importance as a measure of the impact of the solvent resonance on the analyte signal intensity. The WET-180-NOESY sequence achieves a limited solvent signal suppression in the raw data compared to the other tested WET derivatives but the residual chloroform peak width is drastically reduced. This results in an excellent retention of the analyte signals without major distortions. The effect of the signal intensity of the broad feet of the solvent resonance can be observed in the 1D-spectra shown in **Figure 7**. Due to the short repetition times (much shorter than 5 times T_1), it is not possible to obtain the expected integral ratio of 2:2:3 for the respective protons of PS. All our approaches have been designed to yield maximum SNR and displayable analyte signals rather than quantitatively integrable spectra.

WET and its derivatives are suitable pulse sequences to implement instrumental solvent signal suppression in SEC-NMR at 43 MHz using a pulsed z-gradient. For analyte signals in close spectral proximity to the strong solvent signal, WET-180-NOESY is the best technique for unperturbed analysis. However, the 62 MHz spectrometer is not equipped with a gradient coil, which leads to the necessity to find suppression approaches for this instrument without a need for it.

WEFT is a modified version of the *Inversion Recovery* pulse sequence using a combination of a 180° and a 90° pulse separated by a waiting time τ_{WEFT} , with the waiting time adjusted to the apparent T_1 relaxation time of the solvent. The apparent T_1 relaxation time in the WEFT experiment is shortened by measuring in rapid sequence far from equilibrium and, therefore, different from T_1 times in equilibrium. In the case of chloroform suppression, it is necessary to implement a spoil or crusher gradient after acquisition similar to the one in the 90-spoil sequence as the solvent signal FID has not decayed at the end of the acquisition period. Otherwise, the suppression is not sufficient and subsequent mathematical data treatment is severely disrupted. A comparison of SEC-NMR data at 62 MHz for PS in CHCl_3 is given in **Table 6**.

Table 6: Evaluation criteria for solvent suppression in SEC-NMR of PS30k in CHCl_3 (partially overlapping case) at 62 MHz, comparing the WEFT-spoil and the 90-spoil pulse sequence.

Pulse sequence	90-spoil @ 62 MHz	WEFT-spoil @ 62 MHz
Inj. mass polymer [mg]	1.00	1.00
Repetition time [s]	0.50	1.00
$\text{SNR}_A(\text{PS aliphatic})$	39.0	33.0
$\text{SR}_S(\text{CHCl}_3)$, suppression ratio of solvent	142	466(*)
(Raw and Final) $\text{SNR}_S(\text{CHCl}_3)$	(36800) 260	(1900) 79.1
$W_{\text{rem}}(\text{CHCl}_3)$, width of distorted baseline [ppm]	0.24	0.17

(*) $\text{SR}_S(\text{CHCl}_3)$ for WEFT-spoil is calculated using raw solvent SNR with a repetition time of 0.5 s instead of 1 s and is therefore slightly overestimated here.

The WEFT-spoil requires a repetition time twice as long as the 90-spoil, which makes the resulting SNR (without respect to time) not directly comparable.

According to an increase of SNR with the square root of the number of scans, it is expected to be factor a $(1000/500)^{0.5} = 1.41$ higher for 90-spoil, which is approximately the case here, indicating no substantial analyte suppression. The great benefit of this pulse sequence is the combination of a solvent signal suppression in the raw time data prior to digitization by SR_s of up to 20 and very narrow residual solvent linewidths already before mathematical post-processing. After solvent subtraction, the residual solvent SNR is in a comparable range as is expected for the WET pulse sequences. As depicted in **Figure 8** it was possible to achieve a remarkable separation of the aromatic PS signals from the $CHCl_3$ resonance in the resulting spectra, which is due to the pronounced reduction of the “chloroform feet”. Small residual solvent signals remain as $T_{1, \text{solvent}}$ slowly changes over time. This will be tackled in future by usage of a thermoset column compartment to reduce temperature fluctuations in the incoming analyte.

In contrast to the frequency selective approaches like WET and JNR, the WEFT sequence does not require prior optimization of any offsets from the carrier frequency to prevent suppression of analyte resonances close by. The discrimination here is done with respect to T_1 relaxation times, which must be present for this method to work. The ratio $T_{1, \text{polymer}}/T_{1, \text{solvent}} \ll 1$ is true for macromolecules in low molecular solvents (such as in SEC-NMR) but it should be noted that this approach cannot straightforwardly be applied to chromatography of small molecules.[31] However, another appealing feature is the possibility of suppressing more than one solvent signal at a time as long as T_1 for all solvent signals is equivalent. This will be discussed in the following section in more detail.

Figure 8: SEC-NMR results of PS30k in CHCl_3 recorded on the 62 MHz spectrometer using the 90-spoil (left, see Figure 2 (e+i)) and the WEFT-spoil (right, Fig. 2 (a+e+i)) pulse sequence.

Application case 3: Measurement of PS or PEMA in THF - Strong overlap of solvent and analyte bands

The third case discussed herewith is a strong overlapping of the analytes' resonances of interest with a solvent band. This is the hardest problem to tackle. Examples for this case are unfortunately often found in polymer analysis using THF as a solvent, as THF features two broad multiplet resonances. The multiplet centred at 1.84 ppm often overlaps with aliphatic backbone resonances of the polymer and the second multiplet centred at 3.73 ppm coincides with the signals from some functional groups such as aliphatic esters or ethers. Typical examples of this case, therefore, include the aliphatic proton resonances in PS which show on the benchtop spectrometers as a broad band between 1.3 and 2.2 ppm, part of the aliphatic proton resonances in PMMA (0.6 to 2.2 ppm) together with the peak of the $-\text{OCH}_3$ -group at 3.6 ppm or part of the aliphatic proton resonances in PEMA (1.6 to 2.1 ppm). Identification or quantification is therefore not possible without additional information. A broad range of solvent suppression approaches comparing the simple 90-spoil sequences (at 43 and 62 MHz spectrometers) with WET-CP (at 43 MHz) and WEFT(-spoil) at 62 MHz was investigated.

First, SEC-NMR data of PS30k in THF was measured on the Spinsolve 40 and 60 using the 90-spoil pulse sequence and is shown on the top of **Figure 9**. The corresponding results of the numerical evaluation are given in **Table 7**. Please note that due to the change in solvent compared to the previous examples not only the position of the resonances shifts slightly but the elution time of polymer is altered too due to the change in coil swelling of the polymers.

Figure 9: SEC-NMR results of PS30k in THF recorded for different pulse sequences and setups: on the left data is shown which was recorded on the Spinsolve 60 and on the right for Spinsolve 40. The two examples on top were recorded using the 90-spoil sequence (see Figure 2 (e+i)) and on the bottom left using WEFT (Fig. 2 (a+e+h)) and WET-CP suppressing the THF signal at 1.84 ppm only (bottom right, Fig. 2 (b+f+h)). For static spectra of the compounds under investigation see the supporting information.

Table 7: Evaluation criteria for solvent suppression in SEC-NMR of PS30k in THF (strong overlap case) comparing three pulse sequences for solvent suppression used on the 43 MHz and 62 MHz spectrometers. H_A and H_B represent the aromatic ortho- and para- and the meta-protons of polystyrene, respectively.

Pulse sequence	90-spoil @ 62 MHz	90-spoil @ 43 MHz	WEFT @ 62 MHz	WET-CP @ 43 MHz
Inj. mass polymer [mg]	1.00	2.00	1.00	2.00
Repetition time [s]	0.50	0.65	1.00	1.00
SNR_A (Aromatic H _A) @6.7 ppm	83.0	134	69.0	130
SNR_A (Aromatic H _B) @ 6.3 ppm	47.0	69.0	42.0	70.0
SR_S (THF @1.8 ppm), suppression ratio of solvent	205	180	2300(*)	410
(Raw and) Final SNR_S (THF @1.8 ppm)	(82 700) 404	(30 300) 169	(317) 36.0	(38 500) 78.0
W_{rem} (THF @3.3 ppm), width of distorted baseline [ppm]	0.67	1.60	0.31	1.47

(*) The $SR_S(CHCl_3)$ value for the WEFT sequence is calculated using raw solvent SNR with a repetition time of 0.5 s instead of 1 s and is therefore slightly overestimated here.

The results for 90-spoil with signals close to the THF peaks (Figure 9, top left and right) are unsatisfactory on both spectrometers: the signals of the aliphatic protons can hardly be found: shoulders at the THF peak at 1.8 ppm can be seen, which seem to be indicating the presence of the polymer signals but in the contour plot the signals are smeared towards higher elution time. This is less a representation of the initial polymer signals but probably an effect of the slight increase of solvent relaxation caused by the eluting polymer. This is shown as well by the large part of unusable baseline of almost 0.7 ppm. Therefore, these signals are concluded to be unusable for identification or quantification. The aromatic protons are well recovered as they correspond to case 1 and can serve as an estimate of the possible SNR of each sequence.

Surprisingly, the usage of WET-CP (Figure 9, bottom right) suffers from a similar problem, as the polymer signals are not well visible. The THF peak at 1.8 ppm is highly suppressed by this pulse sequence but the polymer resonances in close proximity (by frequency) are suppressed as well and only a weak, elongated artefact remains. Different soft pulse offsets (as discussed in the experimental section) were used but none was found to suppress the solvent with less impact

on the PS signals. For WET, no useful information on the aliphatic protons could be extracted and the width of unusable baseline was similar as above (0.7 ppm).

The WEFT sequence (Figure 9, bottom left) yielded better results. A strong suppression of both THF multiplets by more than a factor of 1000 is achieved while the PS signal can still be partly recovered. The PS signal is not smeared out and has an intensity profile identical to the other PS peaks. This is supported by the much smaller width of unusable baseline (0.3 ppm). However, even with the WEFT method, signals that directly coincide with the strongest part of the THF multiplet are lost. While a full recovery by this method is theoretically possible, in practice however, the THF signal fluctuations are much stronger than the signal of interest and the numerical methods also have limitations. In this case, no spoil pulse addition to the WEFT sequence was found to be necessary as the FID quickly decayed to zero within the acquisition time.

As a second example for the strong overlapping case PEMA in THF is considered. This polymer features a complex spectrum in the aliphatic region with resonances over the range of 0.5 to 2 ppm across the full width of the THF peak at 1.8 ppm and no free standing signals beyond the solvent. It is, therefore, an interesting test of the capabilities of the pulse sequences discussed above. The results of SEC-NMR are shown in **Figure 10** for the pulse sequences 90-spoil and WEFT-spoil.

Figure 10: SEC-NMR results of PEMA78k in THF recorded for different pulse sequences recorded on the Spinsolve 60: on the left by 90-spoil (see Figure 2 (e+i)) and on the right by WEFT (Fig. 2 (a+e+h)).

Table 8: Evaluation criteria for solvent suppression in SEC-NMR of PEMA78k in THF (full overlapping case) at 62 MHz, comparing the WEFT-spoil and the 90-spoil pulse sequence.

Pulse sequence	90-spoil @ 62 MHz	WEFT-spoil @ 62 MHz
Inj. mass polymer [mg]	2.05	2.05
Repetition time [s]	0.5	1.0
SNR_A (PEMA aliphatic @ 1.4 ppm)	93(*)	69
SR_S (THF), suppression ratio of solvent	102	1800
(Raw and Final) SNR_S (CHCl ₃)	(61000) 600	(60) 34
W_{rem} (CHCl ₃), width of distorted baseline [ppm]	0.9	0.3

(*) This value is overestimated due to the only partially suppressed solvent peak close by.

The results for both methods in Figure 10 look similar but some differences are found. In both cases, the remaining solvent signals are quite narrow. In the case of WEFT however, the remaining solvent signal intensity is much weaker. Additionally, the obscured baseline is smaller for WEFT. For WEFT, less and weaker artifacts are found close to the solvent signal position. The WEFT sequence is superior to 90-spoil in this case as well. As in the previous example, the position of the strongest part of the THF multiplets the analyte signals are lost.

Conclusion

This work presents examples for selecting solvent suppression pulse sequences for the online coupling of size exclusion chromatography (SEC) to benchtop ¹H-NMR at 43 respectively 63 MHz. Application examples for solvent suppression

schemes for several narrow molecular weight distributed polymers and typical solvents are shown. The examples are clustered into three groups according to the closeness of the analyte peak of interest to solvent peaks obscuring it (baseline separated, partial overlap, strong overlap). For each group, case studies are conducted and focus on selecting the best pulse sequence for this challenge. The selection is based not only on inspection of the spectra but on a set of objective criteria. These criteria evaluate numerically the solvent suppression in terms of solvent signal reduction, baseline distortion and best possible analyte signal-to-noise ratio. As pulse sequences for solvent suppression, high-field approaches like WET, WEFT and JNR1331 are compared to a simple and efficient one-pulse scheme. A new method is presented to evaluate quantitatively the area of distortion free baseline, which uses the advantage of a 2D data set. High analyte SNR is most important in this method due to the very low concentrations encountered after chromatographic separation.

In the case of baseline separation between signals of interest and solvent signals, a simple one-pulse approach, including a gradient spoil pulse, was found to yield the best results. Solvent suppression before acquisition is sufficient to avoid ADC overflow and this simple method gives the highest repetition rates and consequently the highest analyte signal-to-noise ratio. Upon partial or full overlap between solvent and analyte signals, better suppression by the pulse sequence is required which can be achieved using WET or WEFT schemes. For overlapping signals, WET faces problems at low fields due to its broad suppression window which affects the analyte signals in proximity as well. When using large molecules WEFT has benefits as it can exploit the T_1 relaxation time difference to the solvent small molecules. A direct comparison of WET/WEFT was not included as a 62 MHz spectrometer with z-gradient was not available. At higher fields the advantage can go towards WET, which will be interesting to investigate as better equipment becomes available.

The favourite suppression schemes found in literature for high-field couplings of SEC-NMR or LAC-NMR are dominated by WET. However, for the special application under investigation here, different sequences are utilized with success as well. Different considerations are found to be important at lower fields. Here, obtaining maximum signal for the analyte signals is paramount and the often already broad signals profit less from frequency specific suppression methods.

The online coupling of SEC to ^1H -NMR allows for a uniquely full picture of size distribution and chemical entities in a sample of large molecules (e.g. polymers). The method has been established and optimized in terms of chromatography equipment in previous work,[17,18] while this paper serves as a guideline to use the best pulse sequences for typical analytical problems and shows results which can be achieved. Strong solvent signal suppression can be achieved while retaining a considerable signal-to-noise ratio of the analyte peaks. In future work, the usage of a thermoset column will reduce solvent fluctuations and allow better numerical subtraction. The even higher fields which are available now in benchtop hardware can be used together with gradients. While the evaluation of pulse sequences herein will still be valid, the increased sensitivity and better suppression will allow more information on the compounds under investigation to be extracted.

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Supporting Information

Figure S11: Static NMR spectra of the polymer compounds used in this study as a reference to be used for the on-flow results. The spectra were obtained in 5 mm tubes. Polymers were dissolved in CDCl₃ with concentrations of 34 mg/mL. Number of scans 40, acquisition time 6.5 s, repetition time 15 s. The 'X' mark on the plot indicates a contaminant not part of the sample.