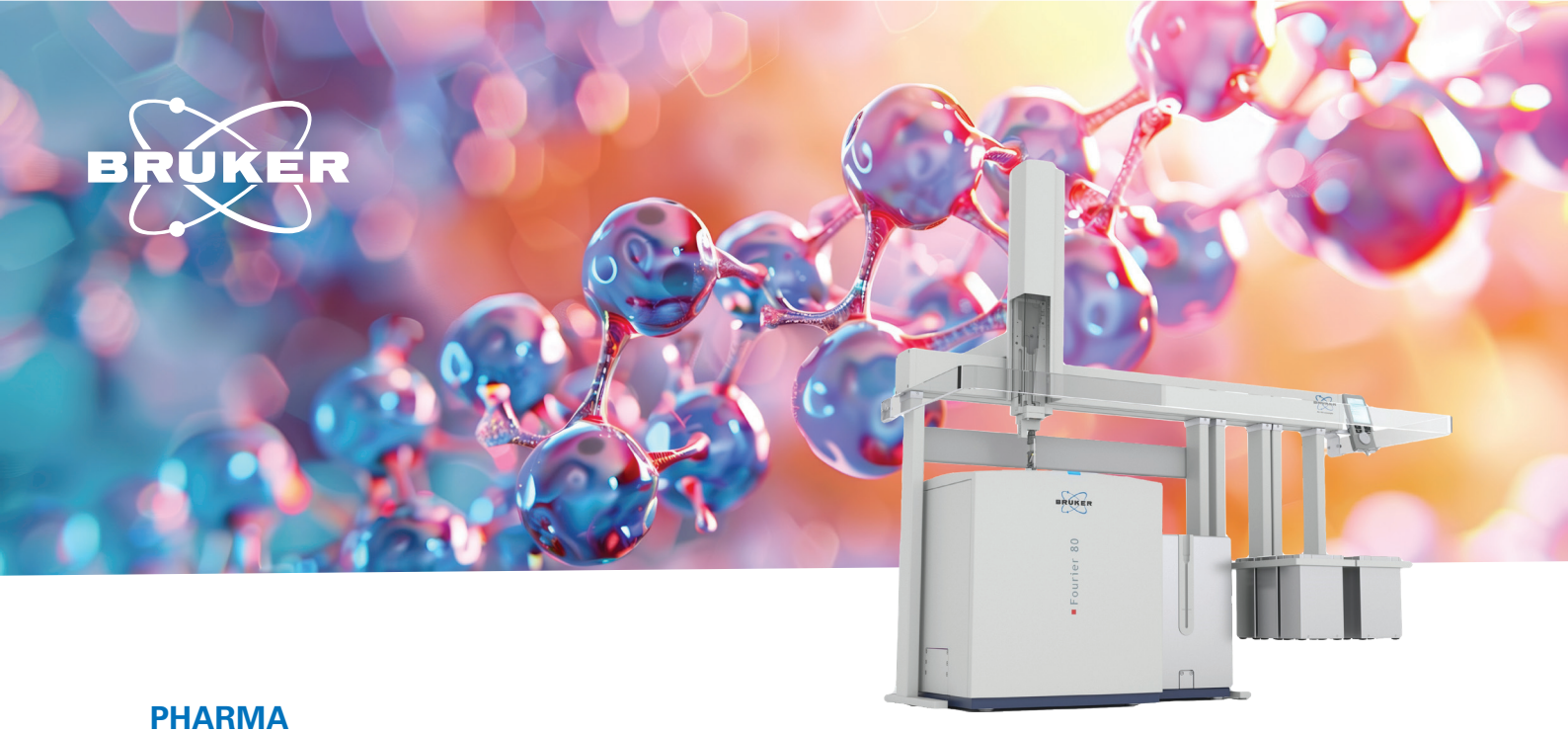

PHARMA

Pharmaceutical Polymer Analysis Using Benchtop NMR: Compendial Testing of Alcohol Ethoxylates and Beyond

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Innovation with Integrity

Ensuring the conformity of polymeric products for pharmaceutical use poses a dual challenge. These materials have inherently complex, statistical structures, requiring tight control of various parameters to guarantee their safety and efficacy. However, the range of available analytical technologies suited for routine polymer analysis is limited. Nuclear Magnetic Resonance (NMR) stands out as one of these few techniques and uniquely offers direct measurement of absolute characteristics. This whitepaper uses the compendial testing of Linear Alcohol Ethoxylates (AEs), an important family of non-ionic surfactants, to demonstrate the capability of NMR for polymer analysis. Specifically, the Bruker Fourier 80 benchtop NMR spectrometer, a compact and cryogen-free system with built-in regulatory compliance support, is highlighted for its capabilities. Designed for Quality Control (QC) laboratories, this instrument provides simple, cost-effective access to NMR technology. It serves as an efficient tool for both qualitative and quantitative testing of organic compounds while offering a powerful toolbox for routine and investigational polymer analysis.

Introduction

Linear Alcohol Ethoxylates (AEs) are a class of non-ionic surfactants widely used in various industries due to their straightforward manufacturing process and customizable properties, such as emulsification, solubilization and wetting. In pharmaceutical applications, AEs play a critical role in enhancing the solubility and bioavailability of poorly water-soluble drugs, a common challenge in drug development. By dispersing hydrophobic compounds in aqueous environments, AEs help improve drug absorption and efficacy. These polymers are also crucial for stabilizing emulsions, suspensions and other colloidal systems, enabling the consistent release and bioavailability of active pharmaceutical ingredients (APIs) in both liquid and solid dosage forms.[1]

[1] See for example: Oyafuso M.H. *et al.* J Nanosci Nanotechnol. **2015**, 817-826; Casiraghi, A. *et al.* Nonionic Surfactants: Polyethylene Glycol Ethers and Fatty Acid Esters as Penetration Enhancers in Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement, **2015** (Springer, Berlin, Heidelberg); Koen S. *et al.* LCGC North America, **2018**, *36(6)*, 385-393 and associated references.

AEs complement other essential non-ionic surfactants used in drug formulations, such as poloxamers[2] and polysorbates. Together, AEs, polysorbates and poloxamers play an essential role in optimizing solubility, stability and the overall effectiveness of pharmaceutical formulations, making them indispensable for modern drug delivery technologies. Additionally, some AEs, such as polidocanol (also known as lauromacrogol), serve as APIs, functioning as local anesthetics.

The generic structure of linear AEs is shown in Figure 1. They are produced on an industrial scale by ethoxylation of natural or synthetic linear alcohols (or a mix of both). The process results in a hydrophilic poly(ethylene oxide) (PEO) polymer chain that is terminated by a linear, hydrophobic alkyl chain, which may be unsaturated. The alcohol chain length and the PEO block can be adjusted to produce a wide variety of highly specific structures that offer precise control over surfactant properties and fine-tuning of the hydrophilic–lipophilic balance (HLB). While the alcohol end groups can be tightly controlled, the PEO block is inherently polymeric, formed through a statistical synthesis process. This leads to variations that can be detected through key parameters, such as number-average molecular weight (M_n), mass-average molecular weight (M_w), and dispersity ($Đ_M$) which is the ratio of M_w to M_n and expresses the breadth of the polymer population distribution.

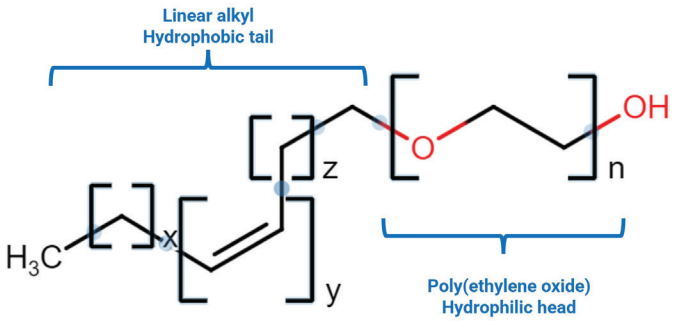

Figure 1: General chemical structure of linear AEs

For pharmaceutical applications, controlling the structure of AEs is critical to ensuring both product efficacy and safety. To obtain a comprehensive actionable insight, a combination of analytical techniques is required. Determining the M_n, M_w, and $Đ_M$ values can be typically achieved using Size Exclusion Chromatography (SEC), also known as Gel Permeation Chromatography (GPC) when the mobile phase is aqueous. However, this method generally provides only relative values. These are based on an empirical calibration scale using standards that differ in chemical nature. Absolute values can only be obtained using the so-called triple detection method, which combines the simultaneous use of refractive index, viscometer, and light scattering detectors. As a result, this approach can be used in development or for investigations but is impractical for most routine controls.

Conversely, Nuclear Magnetic Resonance (NMR) spectroscopy can, in many cases, provide direct and straightforward access to the average number of repetitive units. As such, it can be used to determine the absolute average polymer length and the direct calculation of the M_n value. NMR is a well-established analysis for structural characterization. When it comes to polymers, it is the only analytical method capable of providing detailed insights into composition and microstructure, with the significant advantages of being inherently quantitative and non-destructive.

More precisely, by detecting a specific NMR resonance corresponding to a polymer chain-end, it is possible to directly determine the average number of repetitive units *n* through NMR signal integration. For linear polymers, this directly correlates with the average polymer chain length. The M_n value can then be calculated using Equation 1:

$$M_n = n \times M_{RU} + M_{CE}$$

Equation 1: M_n calculation for linear polymer. *n* = average number of repetitive units as determined by NMR, M_{RU} molecular weight of the repetitive unit and M_{CE} molecular weight of the chain ends.

[2] For more information about the compendial testing of poloxamers by NMR, see: V. Poirier *et al.* Benchtop NMR as a Versatile Tool for Quality Control: Example of Poloxamer Compendial Testing

These general considerations apply to the analysis of virtually any linear polymer and address the otherwise challenging analytical requirements for the structural control of linear AEs. As a result, NMR-based procedures have been incorporated into the United States' Pharmacopeia-National Formulary (USP-NF) for the conformity testing of two specific AEs: Polyoxyl 10 Oleyl Ether (P10OE) and Polyoxyl 20 Cetostearyl Ether (P20CE),[3] whose average structures are shown in Figure 2. Both AEs have individual monographs, and NMR is the compendial method used for determining and controlling their average polymer lengths.

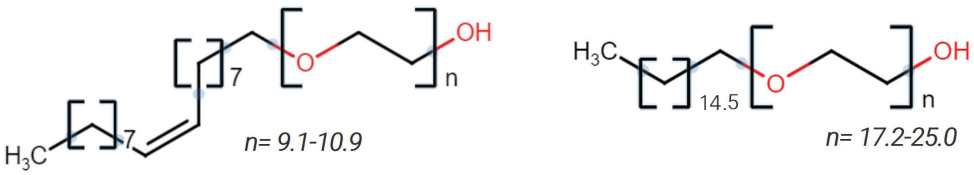

Figure 2: Chemical structures of P10EO (left) and P20CE (right) as well as corresponding specifications for the average polymer length *n*, as indicated in their corresponding USPNF monographs.

It is worth noting that the USP-NF general chapter <313> provides guidelines for determining molecular weight and polymer chain length for linear polypropylene glycol fatty ethers. These are another family of polymeric excipients, where a polypropylene (PPO) block is used instead of PEO. These structures are very similar to linear AEs, and the same general considerations apply for their analytical testing. The USP-NF general chapter <313> requires the use of both SEC and NMR. The first is required for the relative determination of M_n, M_w, and $Đ_M$, while the second is listed for the absolute determination of the average number of repetitive units. Within the section on NMR, it is possible to find detailed guidance on accessing average *n* value on a general basis, although the chapter primarily focuses on two specific products, Polypropylene Glycol 11 Stearyl Ether and Polypropylene Glycol 15 Stearyl Ether.

In all these prescriptive documents, the analytical workflow for NMR testing is identical. It is based on the integration of ^{1}H NMR resonances to determine the average polymer length, ensuring conformity of the PEO block (or PPO in USP-NF <313>). Noteworthily, these tests assume that the fatty termini are as theoretically specified, without fully leveraging NMR's potential to confirm the structure of the fatty block. This despite NMR can be suitable for such analysis as discussed in the penultimate section of this manuscript.

It is important to recognize that only a few compendial tests currently rely on NMR technology. While NMR is well established as a fundamental analytical technique in both academic and industrial research, particularly for synthetic polymers, its adoption in QC laboratories has historically been limited. This is due to the high cost and level of expertise traditionally required for NMR operations. As a result, NMR has typically been seen as impractical for routine compendial procedures.

However, this paradigm is now shifting. Modern spectrometers are simpler to use and can be operated routinely by non-experts, thanks to advancements in user interfaces, fully automated procedures, and simplified maintenance requirements. Recent improvements in GMP compliance for NMR spectrometers and the introduction of benchtop systems have further accelerated this shift. The inclusion of NMR in ICH[4] Q2(R2) guidelines on "Validation of Analytical Procedures" and ongoing revisions of the USP-NF general chapters <761> and <1761>[5] attest to this progress.

In this context, the Bruker Fourier 80 benchtop solution is an ideal tool for QC laboratories, offering the benefits of NMR analysis through a compact, cryogen- and maintenance-free system that supports full compliance with pharmaceutical regulations. This whitepaper discusses how the compendial testing of linear AEs can be easily implemented using the Fourier 80, supporting QC laboratories with a modern, cost-effective alternative to high-field NMR systems while ensuring fit-for-purpose procedures. The document then discusses the straightforward extension of this instrument to other linear AEs and how it can successfully support the control of additional quality attributes using the same procedure. This insight helps illustrate the unique capabilities of NMR and the Fourier 80 for streamlined polymer control.

[3] In the USP-NF monographs, the numerical value in the AEs naming refers to the theoretical average number of ethylene oxide units.

[4] International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use

[5] As of September 2024.

Implementing the USP-NF compendial testing of AEs on the Fourier 80

The NMR testing procedures for P10OE and P20CE described in their respective USP-NF monographs are nearly identical in terms of general instructions and analytical workflow. After melting the product, this must be mixed with deuterated chloroform ($CDCl_3$) in a 1:1 volume ratio, transferred to an NMR tube with the addition of tetramethylsilane (TMS) as a chemical shift reference. Subsequently, the NMR spectrum is ready to be acquired and processed.

It is worth noting that these sections on NMR were developed several decades ago and have not been updated to reflect advancements in NMR technology. In particular:

- The working concentrations specified in the documents are much higher than what is necessary for modern spectrometers, including benchtop models.
- The monographs do not explicitly state proton (^1H) as the nucleus to be recorded, probably because for a long time this was the only one readily accessible, at least for routine NMR.
- The technical specifications for NMR spectral acquisition in the P20CE monograph were written for field-sweep NMR spectrometers, which have been deprecated and replaced by the pulsed Fourier transform technology after its introduction in the 1960s.

Despite the outdated considerations within the monographs, the fundamental principles of the tests remain applicable. Using the described conditions, parameters can be adapted to modern systems based on the general USP-NF chapter <761> and <1761> on NMR.

As stated in the monographs, it is necessary to make sure the NMR acquisition settings can support quantitative analysis. A comprehensive discussion on quantitative NMR is beyond the scope of this manuscript, and readers can refer to the USP-NF general chapters for detailed guidelines. For the purpose of this document, it is important to stress that most of the NMR acquisition variables for accurate quantifications are well understood and known. In addition, they are easily applicable across different procedures and analytes.

When implementing and verifying a quantitative NMR method for the first time on a new analyte, a single key parameter requires experimental determination, the so-called recycle delay. Quantitative NMR indeed relies on achieving a state of equilibrium state between each signal acquisition, making it essential to reach this equilibrium by using an appropriate delay. This recycle delay cannot be predicted and must be determined for each new analyte under a given set of conditions (see Figure 3), such as for P10OE in $CDCl_3$ on an 80 MHz NMR system like the Fourier 80. This delay is set according to the slowest relaxing nucleus used for quantification. It is determined using a dedicated NMR experiment called inversion-recovery, which measures all longitudinal relaxation times (T_1). This allows the calculation of the appropriate recycle delay. This experiment is available in the default library of the Fourier 80 and can be automated. Importantly, it only needs to be performed once during the initial method verification process, as per USP-NF <1226>.

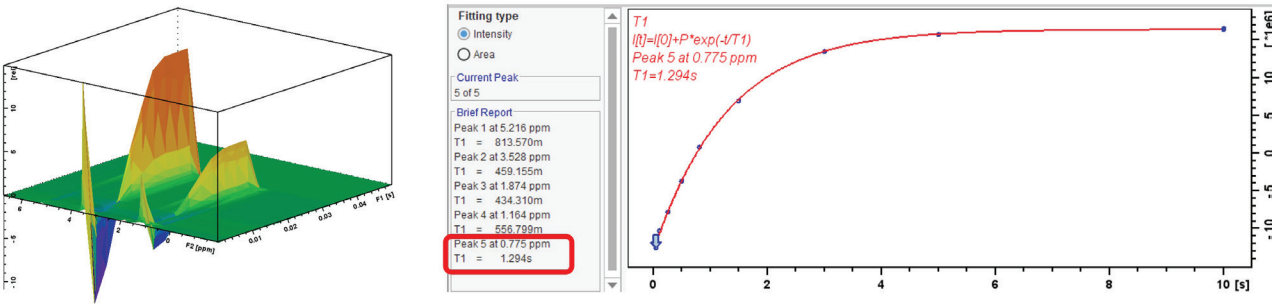

Figure 3: Example of T_1 measurement through an inversion-recovery experiment using the Fourier 80 for P10OE under the USP-NF testing conditions. The image on the left is a 3D representation of the resulting NMR spectral data and the screenshot on the right illustrates the automated calculation of the T_1 for each type of proton in the mixture, allowing the direct identification of the longest value (1.3 s here). Using a recycle delay of at least 5 time, this value ensures suitable conditions for quantitative analysis.

Once this initial verification is completed, quantitative ^1H NMR spectra for P10OE samples can be readily recorded under the conditions specified in USP-NF using the Fourier 80, as shown in Figure 4, along with the corresponding structural assignments.

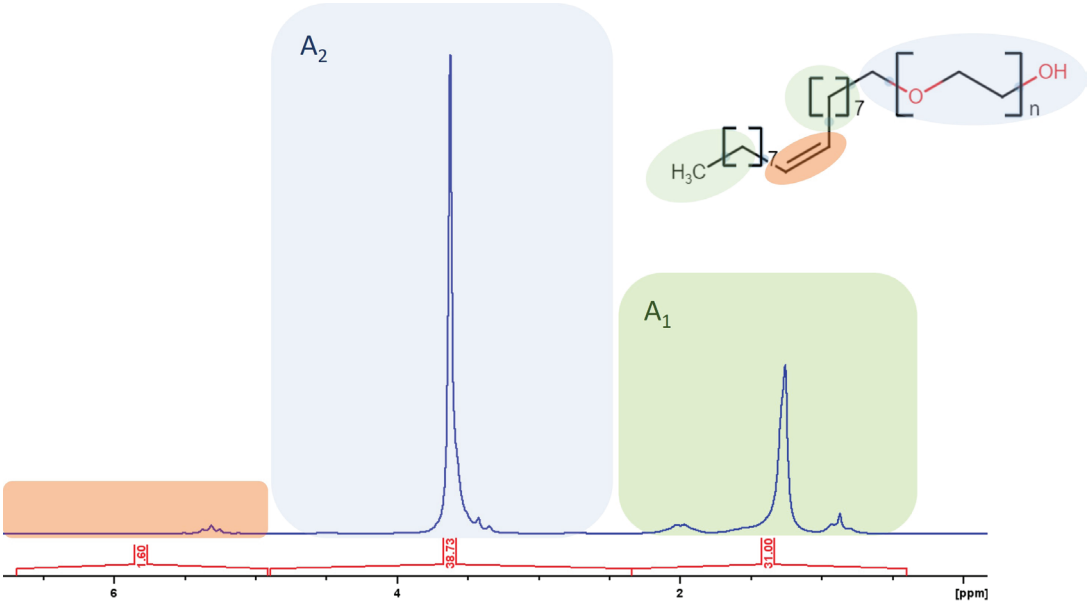

Figure 4: Example of an ^{1}H NMR spectrum for P10OE recorded on a Fourier 80 under the conditions specified in USP-NF. Colored boxes indicate respective structure attributions. Integrations of the A_1 and A_2 areas are used to calculate the average polymer length according to the monograph.

This example demonstrates that the inherently lower resolution of benchtop NMR, compared to high-field alternatives, does not compromise the specificity of the procedure, as the regions to be integrated (A_1 and A_2) remain fully resolved. The integration of these areas thus allows for a straightforward calculation of the average polymer length using Equation 2:

$$n = \frac{31 \times \frac{A_2}{A_1} - 3}{4}$$

Equation 2: Calculation of the average polymer length *n* for P10OE according to USP-NF.

This equation assumes that the fatty chain termination matches its theoretical structure, meaning it contains 31 protons that are "not activated by either oxygen or a double bond". Using this relative reference for area A_1, it is possible to discern that area A_2 accounts for all protons in the PEO block, along with 3 protons from the chain ends. After this correction and normalization by the number of protons in the PEO repeat units (4), the average polymer length *n* is calculated, yielding a value of 8.9 in this example. Importantly, as with any NMR analysis, this absolute measurement is non-destructive and contactless, avoiding any alteration of the polymer distribution, which could occur due to non-specific interactions on an SEC column, for example.

To further assess the suitability of the Fourier 80 for testing P10OE, additional variables were explored to examine their impact on precision and accuracy. These steps are not required for compendial testing but illustrate the typical development and optimization of an NMR-based bespoke method. Two evident potential optimizations could be considered, based on the monograph:

1. Lowering the concentration: As mentioned above, a 50 %v/v concentration may be disadvantageous, resulting in a slurry mixture that complicates sample preparation and is unnecessary from a sensitivity perspective on modern NMR systems. Therefore, a lower concentration of 200 g/L was tested, reducing solution viscosity and simplifying the sample transfer into NMR tubes.

2. Using a carbon-decoupled acquisition sequence ($^{1}H\{^{13}C\}$): By relying on this approach, coupling between protons and the NMR-active ^{13}C isotope, which accounts for 1.1% of the carbons, is suppressed. This merges the side resonances with the main resonances, producing a cleaner baseline. Also, this option is generally beneficial for high-accuracy quantitative NMR applications to help minimize interferences.

Figure 5 shows the typical ^{1}H spectra obtained by adopting these strategies when utilizing the Fourier 80 as well as a comparison with spectra acquired under conventional USP-NF conditions.

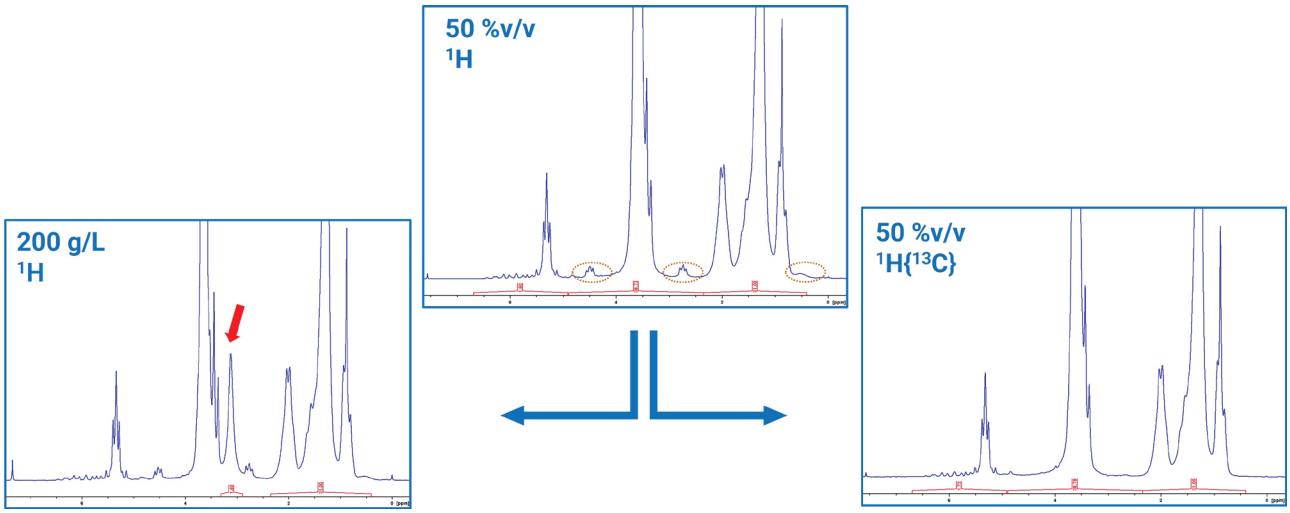

Figure 5: Example of zoomed in ^{1}H NMR spectra of P10OE recorded with a Fourier 80 under USP-NF conditions (top), using a lower concentration (bottom left) and using a $^{1}H\{^{13}C\}$ acquisition scheme (bottom right). The red arrow indicates the ^{1}H resonance corresponding to exchangeable protons resolved when using a 200 g/L concentration. Dotted circles indicate the detectable ^{1}H-^{13}C side resonances, suppressed when adopting a $^{1}H\{^{13}C\}$ sequence.

From the data gathered, it emerges that lowering the concentration results in a shift of the resonance corresponding to the exchangeable protons (indicated by the red arrow in Figure 5). This behavior is to be expected, as the chemical shifts of these protons are sensitive to experimental conditions, such as concentration and temperature. However, this occurrence does not impact the final results, as the resonance remains within the A_2 region, in line with Equation 2.

It is worth noting, though, that the resonance associated to the exchangeable protons accounts for the terminal OH group of P10EO as well as residual water. This comes from the material itself and any solvent used during sample preparation. While this contribution is likely negligible for the selected concentration, it presents a potential source of bias in the original procedure. To address this, additional measures could be undertaken to further separate this resonance from the A_2 region and adjust Equation 2 accordingly. However, any such modification to the concentration specified within the original monograph would likely result in an alternative procedure that requires full method validation, according to USP-NF <1225>.

Conversely, using a $^{1}H\{^{13}C\}$ acquisition sequence does not represent a deviation from the USP-NF monograph for P10OE, as the document does not specify the acquisition sequence. As shown in Figure 5, this alternative approach successfully removes side resonances near the limits of the areas to be integrated. While this does not affect the quantitative result obtained (see below), it can facilitate automated processing and is generally considered good practice for quantitative NMR procedures.

Table 1 summarizes the results obtained for the P10OE batch tested for this implementation study, including replicates. Notably, all results from the Fourier 80 were obtained through fully automated acquisition and processing, demonstrating its ability to offer an ideal workflow for routine QC. Provided suitable conditions are met, as previously discussed, NMR spectroscopy is considered an inherently quantitative analysis. Nonetheless, to definitively validate the results obtained from the Fourier 80, the same P10OE batch was also tested with a 400 MHz NMR spectrometer to compare and confirm accuracy.

Conditions	Result (*n*)	%RSD	Deviation from 400 MHz reference result (%)
50 %v/v, ^{1}H	8.94	0.10	0.3
50 %v/v, $^{1}H\{^{13}C\}$	8.94	0.09	0.4
200 g/L, ^{1}H	8.98	0.05	0.8
200 g/L, $^{1}H\{^{13}C\}$	8.96	0.10	0.5

Table 1: P10EO average polymer length testing according to USP-NF guidelines. Results obtained from a Fourier 80 using the original conditions of the monograph and possible optimizations. 3 replicates were performed for each case and the average data are reported. 400 MHz reference result was obtained on the same batch. All results from the Fourier 80 were obtained in full automation mode (acquisition and processing).

The results presented in Table 1 clearly demonstrate that the Fourier 80 is fully suitable for determining the average polymer length of P10OE:

- The results are virtually identical to those obtained with high-field NMR spectrometers, confirming that the lower resolution and sensitivity of the Fourier 80 do not compromise the performance required for this testing.
- Excellent repeatability was observed across all series, underscoring the high precision achievable with the Fourier 80 in full automation mode.
- Adjusting the concentration or acquisition scheme (e.g., with or without carbon decoupling) does not affect the results. This is to be expected, as NMR is inherently quantitative, and the experimental conditions should not influence the absolute outcome.

It is worth noting that the commercial P10OE batch tested here does not meet the USP-NF criteria (9.1-10.9) - for a formal method verification an USP-NF grade material should be used.

The NMR testing procedure for P10OE can be directly applied to P20CE, as the overall strategy is identical. Figure 6 shows a typical ^{1}H NMR spectrum for P20CE recorded on the Fourier 80 under USP-NF testing conditions. The spectrum closely resembles that of P10OE, with the main difference being the absence of resonance in the unsaturation region (highlighted in red in Figure 4). Thus, the same analytical procedure can be applied,[6] with only the final equation requiring adjustment to account for structural differences. This modified relationship is presented in Equation 3.

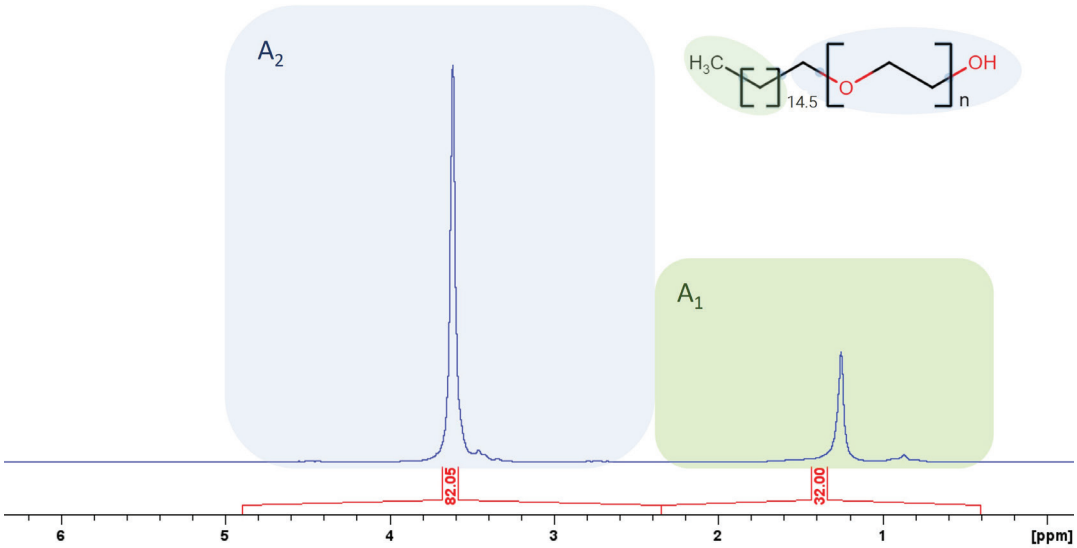

Figure 6: Example of ^{1}H NMR spectrum of P20CE recorded with a Fourier 80 under the USP-NF conditions. Colored boxes indicate the structure attribution. Integrations of the A_1 and A_2 areas are used for the calculation of the average polymer length in accordance to the monograph.

$$n = \frac{32 \times \frac{A_2}{A_1} - 3}{4}$$

Equation 3: Calculation of the average P20CE polymer length *n* according to USP-NF

For P20CE, the linear alkyl chain is fully saturated, corresponding on average to a cetostearyl terminus. As outlined in the monograph, the average polymer length *n* is calculated considering 32 protons "not activated by oxygen" while applying the same other considerations as for Equation 2.

[6] After verification of the longest T_1 of the new product under testing conditions. In the case of P10EO and P20CE, because of their very similar structure, no significant change is expected. This was experimentally verified during this study.

The variations in concentration and acquisition sequences explored for P10OE are applicable to P20CE and can be compared with reference result obtained with a 400 MHz NMR spectrometer, as summarized in Table 2. Conclusions similar to those presented for P10OE can be drawn. More precisely, excellent precision and accuracy were consistently observed, with no significant difference between the reference results obtained with high-field NMR instruments and those calculated from data collected with the Fourier 80. This is valid regardless of the concentration or acquisition scheme used.

Conditions	Result (*n*)	%RSD	Deviation from 400 MHz reference result (%)
50 %v/v, ^{1}H	19.8	0.07	0.8
50 %v/v, $^{1}H\{^{13}C\}$	19.7	0.06	0.4
200 g/L, ^{1}H	19.8	0.08	0.8
200 g/L, $^{1}H\{^{13}C\}$	19.7	0.10	0.7

Table 2: Results obtained with a Fourier 80 for the average polymer length testing of a P20CE batch according to USP-NF using the original conditions of the monograph and possible optimizations. 3 replicates were performed for each case and average values were calculated. 400 MHz reference result was obtained on the same batch. All results from the Fourier 80 were obtained in full automation mode (acquisition and processing).

These results demonstrate the suitability of the Fourier 80 to perform compendial testing of both P10OE and P20CE according to USP-NF, offering a simpler and more cost-effective alternative to high-field NMR systems. They also highlight a key advantage of NMR for QC: a single procedure can be designed and applied to multiple products with minimal changes.

This concept, known as the "platform procedure," was introduced in the recent ICH Q2(R2), ICH Q14, and USP-NF <1220> as part of the Analytical Quality by Design (AQbD) framework. It provides significant flexibility, simplifying the analytical procedure portfolio. With appropriate justification, validation efforts can be significantly reduced, and lifecycle management streamlined when deploying platform procedures.[7]

The examples of P10OE and P20CE testing, even if originally performed for compendial purposes, constitute an excellent example of a possible platform procedure approach. In effect, the NMR analytical procedures in the two respective monographs are nearly identical, differing only in the final calculation. This capability perfectly fits the definition of a platform procedure, *i.e.* a single procedure to test quality attributes of different products without significant changes.[8] Most importantly, this procedure can be easily extended to other linear AEs for bespoke testing, further expanding the scope and the ability to streamline operations with such approach. The broader application of this procedure to other AEs is discussed in the next section.

Extending USP-NF compendial procedures to other linear AEs for average polymer length

The procedures described in USP-NF for P10OE and P20CE can be extended to determine the average polymer length (*n*) of the PEO block in other linear AEs as well as to calculate the corresponding M_n. While this last metric is not part of the original compendial procedure, it can be directly assessed using Equation 1. The only requirement is knowing the fatty chain-end, which is key to calculate *n* based on NMR experimental data, as outlined in Equation 4.

$$n = \frac{\mathrm{N} \times \frac{A_2}{A_1} - 3}{4}$$

Equation 4: General calculation of the average polymer length *n* of AEs with N being the number of proton non-activated by oxygen or unsaturation, e.g. the number of protons in the A_1 area.

Figure 7 illustrates how this procedure can be extended to four other commercial linear AEs, each with different chain-end structures and PEO block lengths. For example, Polyoxyl 20 Oleyl Ether (P20OE) is a direct homologue of P10OE, differing only in the PEO block average length. In contrast, Polyoxyl 10 Stearyl Ether (P10SE) and Polyoxyl 10

[7] For a detailed discussion on platform procedures see: V. Poirier, Combining Robustness & Flexibility - Benefits & Examples of using Benchtop NMR for Simplified Design & Risk-assessment of Analytical Quality Control Procedures

[8] ICH Q14 definition for platform procedure is: "An analytical procedure that is suitable to test quality attributes of different products without significant change to its operational conditions, system suitability and reporting structure."

Tridecyl Ether (P10TE) have different linear alcohol chain-ends. Lastly, Polyoxyl 9 Dodecyl Ether (P9DE), also known as polidodecanol or Lauromacrogol 400, varies in both its theoretical PEO average chain length and its fatty terminus. Despite these variations, the overall structure remains consistent, as demonstrated by the similar ^{1}H NMR spectra. As a result, the same platform procedure, adapted from the USP monographs, can be used to control the average polymer length of these AEs.

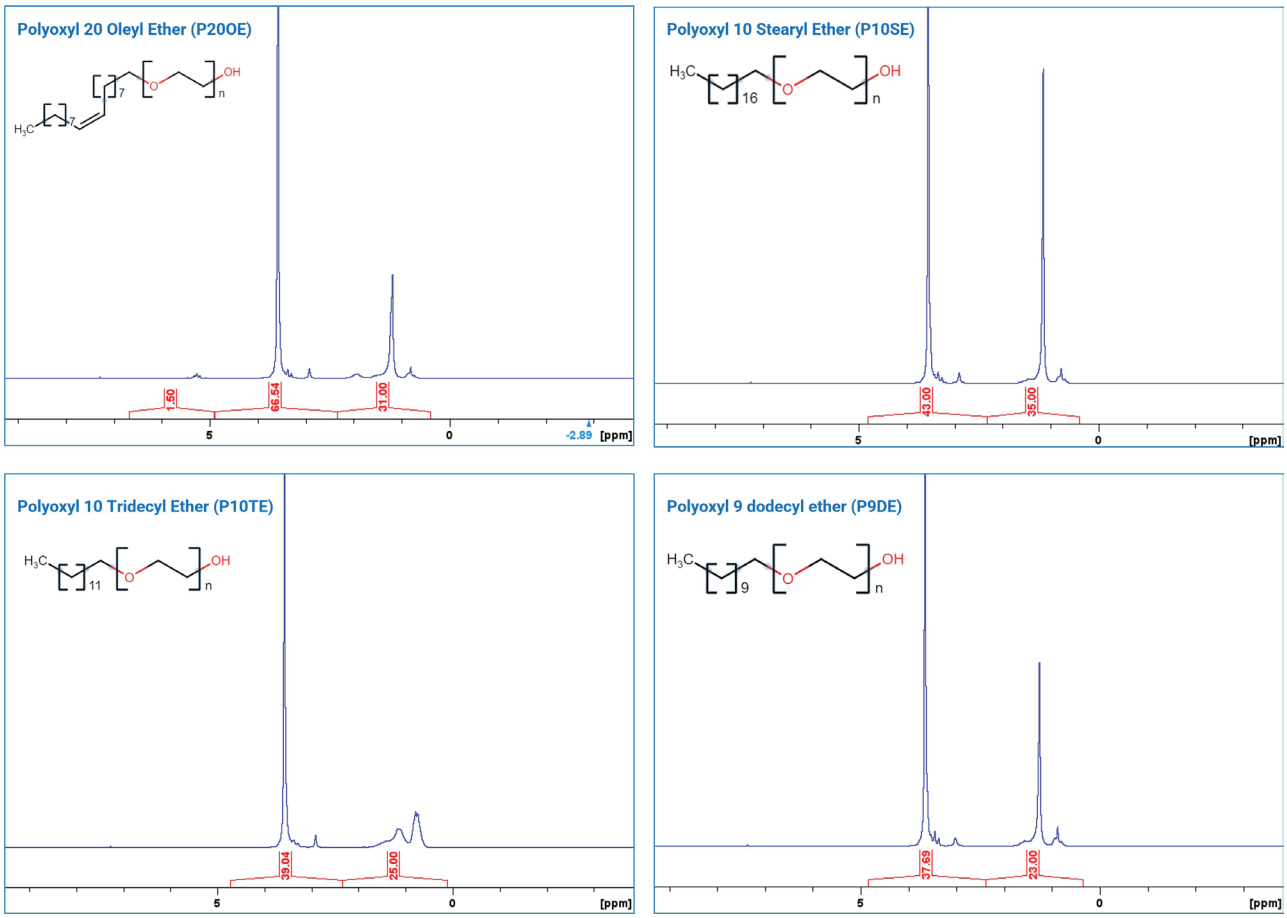

Figure 7: Example of ^{1}H NMR spectra of four AEs recorded with the Fourier 80 using a platform procedure adapted from the USP-NF testing for P10OE and P20CE, next to the polymers' corresponding theoretical structures. For P10TE, the fatty linear chain end is a mixture of C11-C12 with C13 dominating.

Table 3 presents the results obtained for all the batches investigated within this study. Notably, similar to the P10OE batch discussed in the previous section, the P20OE batch showed a significant deviation from its theoretical number of EO units (20), with an experimental *n* value of 15.9. In contrast, all the other products remained within 10% of the theoretical values indicated by their names.

Sample	Batch	N	M_{CE} (g/mol)	Experimental results	
				n	M_n (g/mol)
P20OE	1	31	268.5	15.9	968
P10SE	1	35	270.6	10.0	711
P10TE	1	25	200.4	9.01	597
P9DE	1	23	186.4	8.67	568
	2	23	186.4	8.62	566

Table 3: Results obtained with the Fourier 80 from testing the average polymer length testing of various commercial AEs batch using a platform procedure adapted from the USP-NF monographs for P10OE and P20CE. The average polymer chain length was calculated according to Equation 4, while the corresponding M_n was obtained using Equation 1, based on the N and M_{CE} values specific for each structure.

P9DE is unique within this product family, as it is an API with its own monograph (2046) in the European Pharmacopoeia (Ph. Eur.). This standard also uses NMR but unlike the USP-NF monographs for P10OE and P20CE, which rely on theoretical assumptions about the fatty block, the Ph. Eur. procedure requires complete control of the P9DE structure the control of both the average PEO block length and the fatty acid chain length.

The Ph. Eur. testing approach uses quantitative ^{13}C NMR, which is considerably more challenging than ^{1}H. While ^{13}C NMR provides much more detailed information about the polymer microstructure (see example in the next section), it is more time-consuming to implement. Quantitative ^{13}C NMR indeed requires significantly longer acquisition times due to the limited natural availability of ^{13}C. In addition, ^{13}C NMR spectroscopy involves longer recycle delays compared to ^{1}H, due to inherently longer relaxation time for carbon nuclei. This results in experiments that can take several hours per sample. In contrast, quantitative ^{1}H NMR procedures only take a few minutes and, in the specific case of P9DE, is likely to provide all the required information. This aspect is discussed in the next section.

Expanding the NMR analysis of AEs using the Fourier 80

Structural controls using ^{1}H NMR

The QC strategy presented in the USP-NF monographs for P10OE and P20CE relies on the theoretical structure of the linear alkyl chain to measure the average chain length of the PEO block. However, ^{1}H NMR on linear AEs can provide additional quantitative information that can expand the control of the PEO block and the fatty chain-end structure.

A clear example is the ability to measure the level of unsaturation in the linear alkyl chain. Figure 8 shows a zoomed-in ^{1}H NMR spectrum of the P10OE batch studied in the previous sections. It shows the presence of unsaturation (also visible in P20OE, see Figure 7). However, it is notable that:

1. One main resonance dominates, but several smaller resonances are present, indicating the presence of different microstructures.
2. The ratio between the aliphatic area (A_1), normalized by the theoretical number of protons (31), and the unsaturated area is lower than expected. The theoretical content of protons from double bonds is 1.6 instead of the expected value of 2 for a "perfect" oleyl chain

These two observations suggest that the chain termini are not purely oleyl chains but rather a mixture of saturated and unsaturated alkyl, with an unsaturated-to-saturated ratio of approximately 8:2. This is not unexpected, as even USP-grade oleyl alcohol can contain up to 14% saturated isomers and 8% unsaturated isomers. However, this finding implies that the results calculated using Equation 2 are slightly biased, as the value of 31 should be adjusted to 31.4. In this case, the error is negligible, affecting the *n* result by only around 1%.

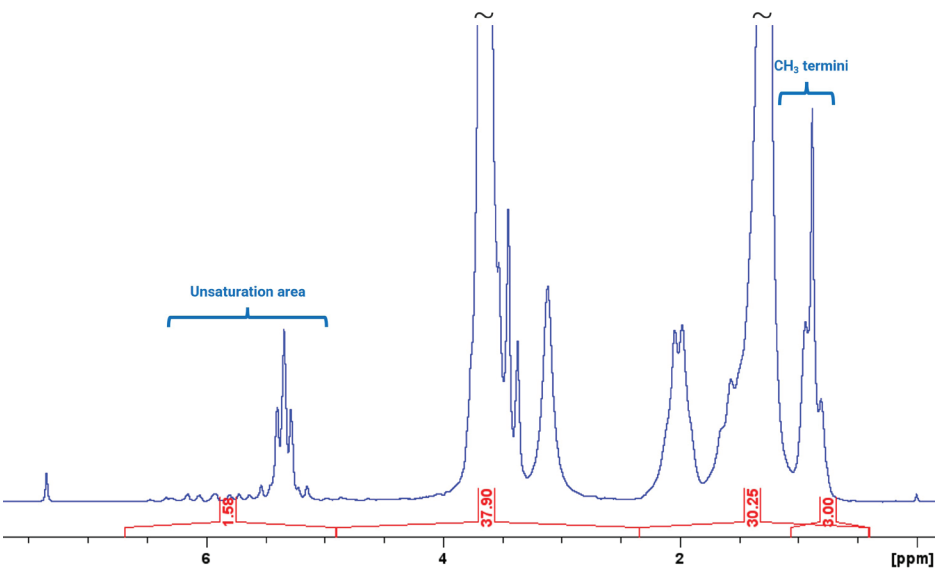

Figure 8: Zoom of the ^{1}H NMR spectrum of a P10OE sample recorded with a Fourier 80 using derivative conditions of the USP-NF monograph ($^{1}H\{^{13}C\}$, 200 g/L).

These considerations are valid solely if the average length of the fatty termini corresponds to the theoretical value, e.g. a C18 chain for P10OE. The Fourier 80 can support the direct control of this parameter through the selective detection of the CH_3 termini of the fatty chain, as illustrated in Figure 8. By integrating this resonance and comparing it to the A_1 as well as unsaturated areas, the average fatty chain length can be accurately determined. In this example, the average length is closer to a C17 chain, which in turn affects the calculation of the average PEO block length, resulting in an *n* value of 8.7.[9]

Importantly, the results obtained with a Fourier 80 are identical to those obtained using a 400 MHz spectrometer (Figure 9). This demonstrates that the proximity of the CH_3 and the main A1 signals does not compromise accuracy when recorded on the Fourier 80. Therefore, the Fourier 80 is well-suited for the tracking and analysis of AEs, including their fatty chain components.

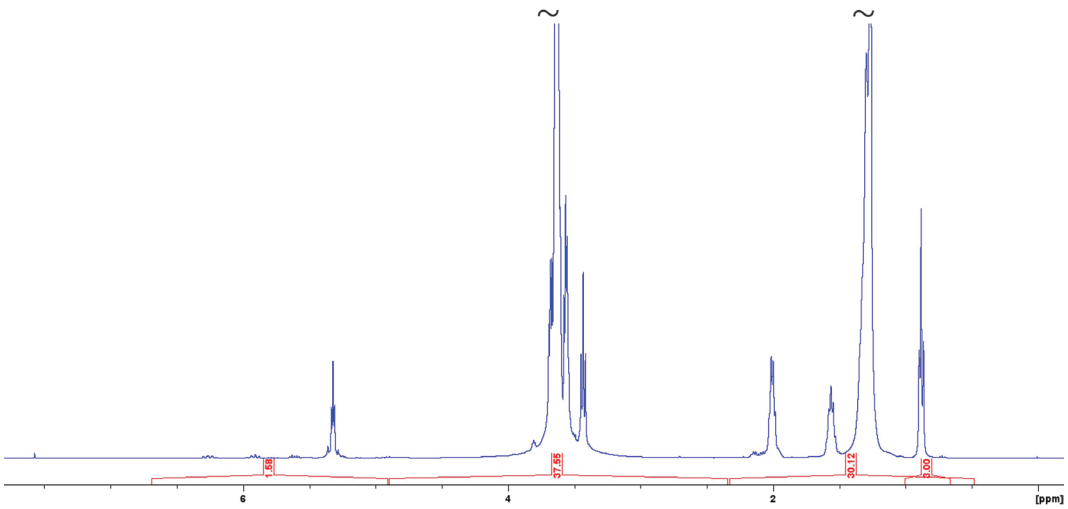

Figure 9: Zoom of the ^{1}H NMR spectrum of a P10OE batch recorded with a 400 MHz AVIIIHD system under experimental conditions comparable to the analysis carried with a Fourier 80.

These considerations apply to any linear AEs where the CH_3 chain-end can be specifically detected, including P9DE. Instead of relying on a more complex, time-consuming quantitative ^{13}C approach, ^{1}H NMR could provide the necessary metrics for analytics required by Ph. Eur. 2046. Indeed, the CH_3 chain-end of P9DE is clearly detectable using the Fourier 80 (as seen in Figure 7), supporting the direct measurement of the fatty chain length, similarly to P10EO. For the two batches of P9DE studied here, this analysis results in the expected C12 chain length. This confirms the *n* values shown in Table 3 and, as such, meet the criteria from Ph. Eur. while avoiding a time-consuming ^{13}C-based procedure. Adopting such a cost-efficient ^{1}H-based approach would however represent an alternative procedure and would therefore require formal validation for use in compendial testing.

[9] In this example, by normalizing the CH_3 termini to 3, the A_1 area integrates for 30.25, accounting for this CH_3 and thus 13.6 CH_2 to which add 1.6 unsaturated carbon and one CH_2 inherently present in the A_2 area. This yields a total of 17.2 carbons on average for the fatty termination. Then the A_2 area directly yield *n*, considering the 3 protons not due to the PEO block and that the EO repetitive unit contains 4 protons, see Equation 4.

Structural characterization for investigations

In addition to routine control, NMR spectroscopy is the gold standard for structural investigations and elucidation. This applies to the Fourier 80 too, which can be used for the extensive range of NMR experiments originally developed for high-field systems. The Fourier 80 has a global topology similar to its high-field counterparts and can be equipped with multinuclear and gradient capabilities. Moreover, it runs on the same software as all Bruker NMR spectrometers (Topspin), allowing one-dimensional and multi-dimensional experiments to be performed just as they would on high-field systems, using the same experiment libraries. This ensures seamless transfer between systems based on specific needs. For linear AEs, the resolution and sensitivity offered by the Fourier 80 are adequate for structural investigations, such as the analysis of an Out-Of-Specification (OOS) result. While a detailed discussion of NMR experiment selection and interpretation for structural analysis is beyond the scope of this manuscript, Figure 10 illustrates the wealth of information that can be gathered. Using the 200 g/L P10EO sample preparation procedure from previous sections and standard experiments provided in Topspin, detailed information was recorded in less than an hour, enabling the complete assignment of the carbon spectrum. This analysis confirmed the overall structure of the P10EO sample, validating that the OOS result for the *n* value was indeed due to a shorter-than-required PEO block, with no other significant structural defects detected.

This example demonstrates the versatility of the Fourier 80 as a valuable qualitative investigative tool for QC laboratories, enabling structural investigations using the same equipment used for routine tests, without any need for special conditioning. This is especially relevant for polymer analysis, where the number of available technologies for quantitative analysis is limited compared to those at hand for well-defined molecules, due to the inherent complexity of polymers.

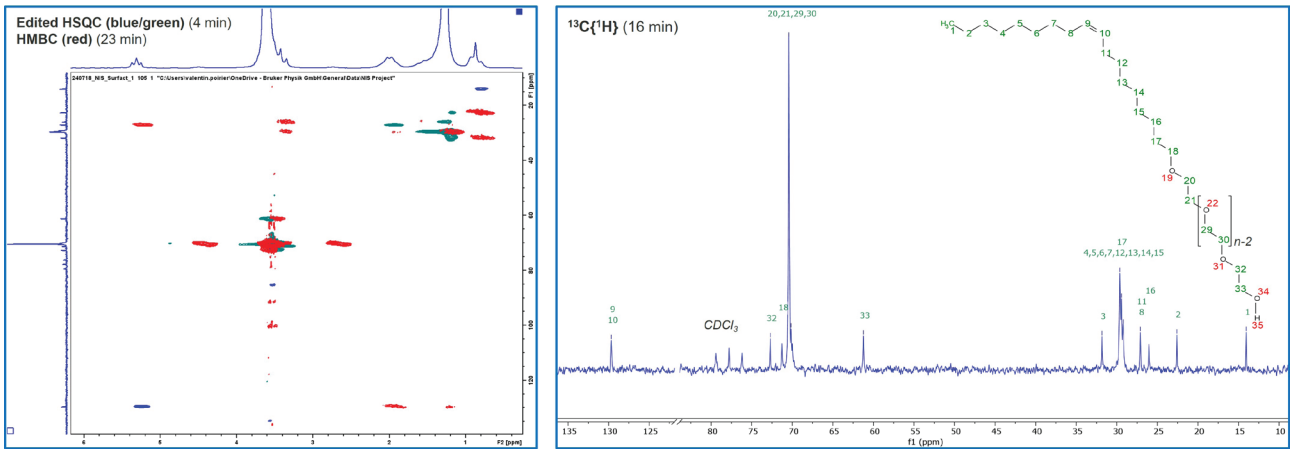

Figure 10: Example of possible structural investigations via NMR using the Fourier 80 on P10OE (200 g/L in $CDCl_3$): overlay of Edited-HSQC and HMBC spectra (left) and $^{13}C\{^1H\}$ spectrum (right) for the full attribution of carbon resonances.

Conclusion

NMR spectroscopy provides invaluable information for the characterization of organic polymers, and easily delivers quantitative data for the QC of these materials. In this whitepaper, we demonstrated that the benchtop Fourier 80 NMR offers a cost-efficient instrument for the compendial testing of P10OE and P20CE in line with USP-NF standards. Using the same methodology, it is possible to set up effective and precise control over a range of linear AEs. In addition, it offers a key solution to easily measure additional polymer characteristics that are not covered by the original monographs with minimal modifications. These opportunities clearly highlight the advantages of NMR and the Fourier 80 for QC, as these support recent platform procedure concepts, simplifying and streamlining analytical procedure management.

Linear AEs are just one example of materials that can benefit from NMR as an efficient analytical tool. While this is a critical technology for polymer analysis, as evidenced by other compendial tests,[10] NMR spectroscopy is overall one of the most versatile and robust analytical techniques available for organic compounds. It has many applications in QC, from efficient identification testing to high-accuracy assays.[11] Additionally, it offers a powerful tool for investigating OOS results, supporting a rapid examination of mixtures as well as the identification of unknown products using one single piece of equipment.

The Fourier 80 benchtop NMR instrument delivers these inherent benefits within a compact, cryogen-free system that requires virtually no maintenance. With built-in GMP support to help laboratories ensure regulatory compliance and full automation capabilities, it offers an ideal, cost-efficient solution for laboratories to integrate and maximize the benefits of NMR as a modern technology for QC.

Acknowledgements

Cristina Daolio Diehlmann, Nicole Eckstein and Birk Schuetz

[10] See for example ref. 2 and V. Poirier, Adapting and Streamlining Compendial Procedures with the Fourier 80 Benchtop NMR Spectrometer: Examples of the USP-NF and Ph. Eur. Betadex Testing, July 2024

[11] See for example Bruker's collection of whitepapers on the application of NMR for QC using the Fourier 80: Benchtop NMR for quality control applications | Bruker

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