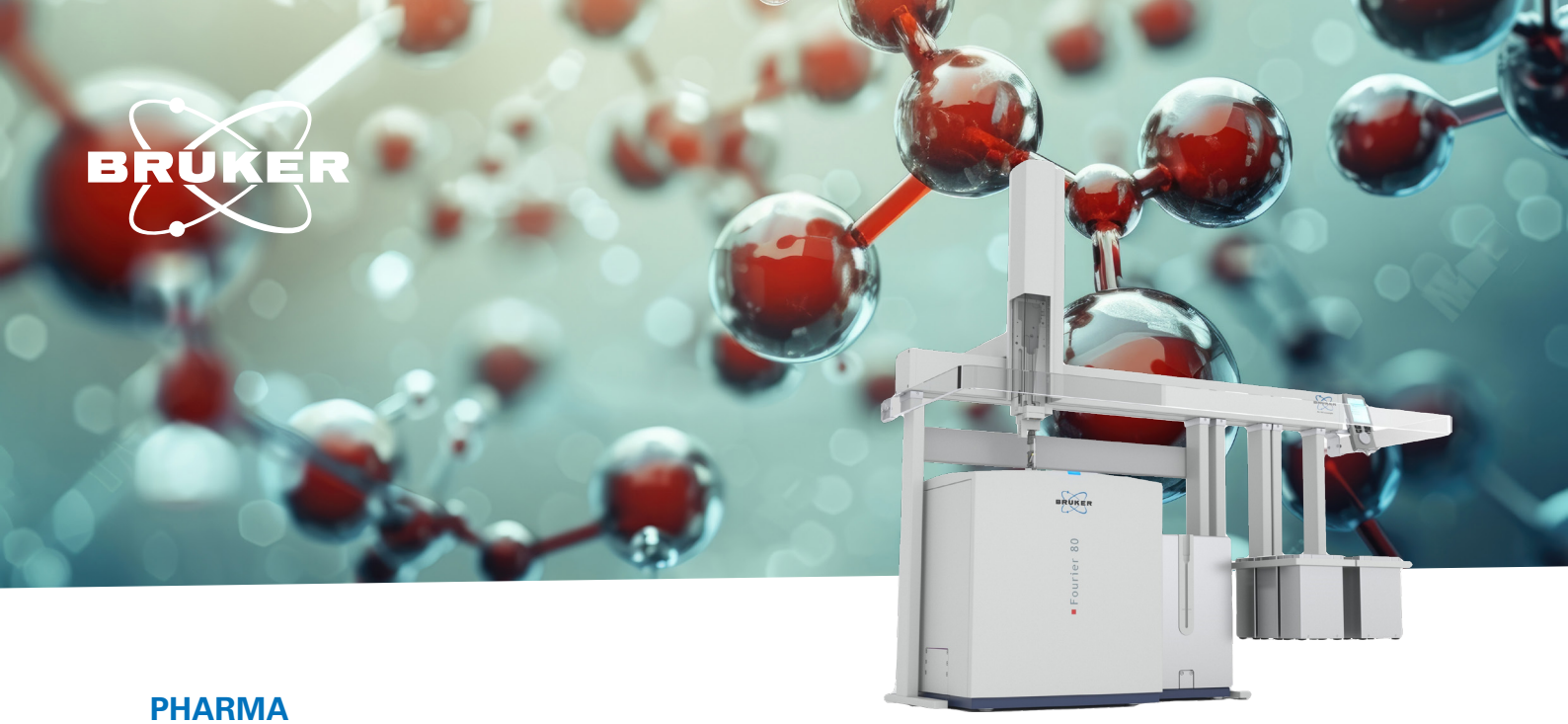

PHARMA

Simplifying Quality Control of PLGA and Synthetic Copolymers

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Innovation with Integrity

Poly(lactide-co-glycolide) copolymers (PLGAs) are biodegradable and biocompatible synthetic material that are essential in multiple pharmaceutical applications. These copolymers, derived from lactic acid and glycolic acid, degrade into non-toxic metabolites, making them ideal for drug delivery systems and resorbable implants. Thanks to the ability to finely control PLGAs' composition and microstructure during manufacturing, it is possible to fine-tune their properties for targeted applications. The wide range of PLGA-based materials available can make quality consistency particularly challenging. This can have consequences when it comes to polymer performance and safety. Although widely used in approved pharmaceuticals, PLGAs have only recently been included in key compendia, and only with a limited number of products.

This paper discusses the challenges of standardizing and controlling the quality of PLGAs. It showcases the key role of Nuclear Magnetic Resonance (NMR) spectroscopy in addressing these issues. In particular, the document discusses the capabilities of a benchtop NMR system, such as Bruker's Fourier 80 NMR, in the delivery of accurate and reproducible characterization for PLGAs. More precisely, this study demonstrates that this compact, cryogen-free NMR system can effectively determine the critical ratio of lactide (LA) and glycolide (GA) units in PLGAs, offering a cost-effective alternative to high-field NMR systems for routine quality control. Even more, the study presents how this is only one of the many opportunities offered by a benchtop NMR system.

Introduction

PLGAs are synthetic thermoplastic polyester copolymers that are widely utilized in pharmaceutical applications due to their biodegradability and biocompatibility. Derived from lactic acid and glycolic acid, these polymers are hydrolyzed in vivo into non-toxic metabolites, making the materials suitable for a wide range of biopharmaceutical applications. Besides, they exhibit properties that can be finely adjusted by controlling their microstructure. As a result, it is possible to develop materials with specific degradation rates and mechanical characteristics, which are critical for pharmaceutical applications, such as drug delivery systems and resorbable implants.[1]

The generic structure of PLGAs is shown in Figure 1. On an industrial scale, they are generally produced by ring-opening polymerization (ROP) of the cyclic lactide (LA) and glycolide (GA) monomers. While many catalysts have been developed and still constitute a focus for fundamental and applied research, a tin-based catalytic process remains dominant in large-scale, commercial manufacturing. This is due to the process' ability to offers robust synthesis of PLGAs, where the ratio between the two blocks can be controlled directly by varying the monomer feeds. In addition, the chain ends are determined by the catalytic initiator, and the average copolymer length can be tuned via the ratio of the initiator to the monomers.

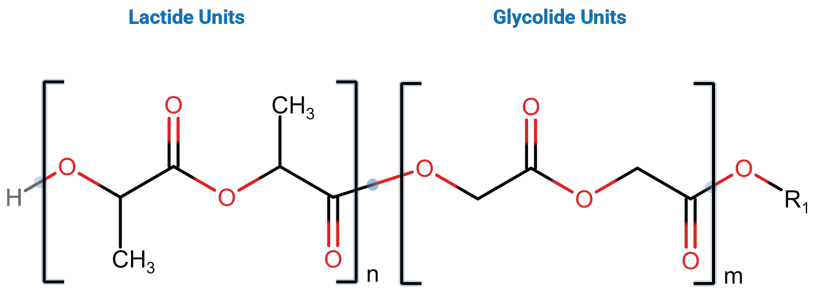

Figure 1: Generic chemical structure for PLGAs. R1 is usually H (acid termini) or a short aliphatic chain (ester termini).

The ability to finely control and modify PLGA structures during the manufacturing process is a key reason behind the commercialization of a wide range of PLGAs and variants. These include highly sophisticated structures as well as PLGA-derived materials that feature other cyclic esters, such as ε-caprolactone. By varying the chain length, chain ends, monomer distribution (including block or random arrangements), and the tacticity of the poly(lactide) blocks, the physicochemical properties of the material, can be precisely tuned. Examples include the hydrolytic degradation, crystallinity, and interaction with active pharmaceutical ingredients (APIs).

Accurate and reproducible control over these parameters is essential to ensuring consistent performance in drug delivery formulations, whereby the release profile must be predictable over extended periods. This requirement poses challenges for the standardization and quality control of PLGAs for pharmaceutical use, as their complex microstructure must be accounted for to ensure consistent product quality and performance.

This issue contributed to the slow inclusion of PLGA in pharmacopeias and the delayed acceptance of generics for PLGA-based products. While more than 55 drug formulations containing PLGAs are already on the market, the FDA's research program on PLGAs was only published in 2021.[2] In addition, the inclusion of PLGA monographs in the United States Pharmacopeia–National Formulary (USP–NF) is not expected until May 2025.[3] This delay follows extensive effort by the USP–NF to harmonize nomenclature and testing methods to control PLGAs for "USP–NF grades," as reported in a stimuli article.[4]

This document suggested the development of both individual and umbrella monographs for PLGA-related products, potentially offering significant flexibility. However, currently, only individual monographs will become official. These will cover a small subset of PLGA-based excipients, which are listed in Table 1. These excipients are restricted to products with a 50:50 molar ratio of LA to GA blocks (referred to as molar LA:GA ratio hereafter), specific average molecular weight ranges, and defined termini.

[1] See for example Operti *et al.* Int. J. Pharma. **2021**, *605*,120807; Siegel *et al.* Polymers (Basel) **2011**, *3(3)*,1377; Elmowafy *et al.* J. Pharm. Investig. **2019**, *49*, 347; Song *et al.* Drug Des Devel Ther. **2018**, *12*, 3117 and associated references.

[2] Xia *et al.* AAPS J. **2021**, 23, 92.

[3] Last drafts were published in USP-PF 48(3). Final version will become official May 1st, 2025.

[4] Zhang *et al.* Zhang "A Practical Approach to Compendial Nomenclature and Testing For Lactide and Glycolide Polymers and Related Polymeric Excipients", USP-PF 49(6).

Entry	Harmonized Name	Termination	Molar LA:GA ratio	Weight-average molecular weight (M_w) (g/mol)	Inherent Viscosity (dL/g)
A	dl-Lactide and Glycolide (50:50) Copolymer 12000 Acid	Acid	47:53 to 53:47	7,000 to 17,000	0.16 to 0.24
B	dl-Lactide and Glycolide (50:50) Copolymer 12000 Ethyl Ester	Ethyl Ester	47:53 to 53:47	7,000 to 17,000	0.16 to 0.24
C	dl-Lactide and Glycolide (50:50) Copolymer 46000 Acid	Acid	47:53 to 53:47	36,000-57,000	0.45 to 0.60

Table 1: PLGA references listed in USP-NF as excipients as of 1st May 2025.

The Chinese Pharmacopoeia (ChP) took a more pioneering approach, with monographs for PLGAs added in its 2020 edition and more inclusive definitions. As shown in Table 2, the scope for possible PLGA excipients is significantly wider compared to the USP–NF, with several molar LA:GA ratios listed. In addition, chain ends are not specified, and the average molecular weight is indirectly controlled via the inherent viscosity of the product, allowing for a wide range of acceptable values.

Entry	Harmonized Name	Molar LA:GA ratio	Inherent Viscosity (dL/g)
A	Poly(lactide-co-glycolide)(5050)	45:55 to 55:45	0.1 to 1.0 (indicated value ±10%)
B	Poly(lactide-co-glycolide)(7525)	70:30 to 80:20	
C	Poly(lactide-co-glycolide)(8515)	80:20 to 90:10	

Table 2: PLGA references listed as excipients in the 2020 edition of the ChP.

Assessing the quality and conformance of such sophisticated copolymers for pharmaceutical use requires a set of analytical techniques. While simple solutions, such as viscosity measurements can help determine bulk properties, controlling the product at the structural level requires more advanced analytical technologies. Generally, this is achieved by a combination of Size Exclusion Chromatography (SEC), also known as Gel Permeation Chromatography (GPC), and Nuclear Magnetic Resonance (NMR) spectroscopy.

SEC/GPC is indeed the most common technique to determine the average molecular weight of polymers, both in terms of weight (M_w) and number (M_n). However, it generally only delivers relative values, as it is based on an empirical calibration scale that uses standards that may differ in the chemical composition. For absolute results, it is necessary to use simultaneous refractive index, viscometer and light scattering detectors. As a result, absolute measurement is impractical for most routine controls.

Conversely, NMR is a well-established analytical technique for structural characterization. When it comes to polymers, it is the only analytical method capable of providing detailed insights into their composition and microstructure. Additional significant advantages include its inherently quantitative and non-destructive nature. NMR provides direct and precise information about various polymer structural properties, such as the direct and exact confirmation of the nature of the polymer, quantitative determination of block ratios, and the detection of residual monomers. For linear polymers with a controlled structure, NMR can also provide the average number of repeating units by specifically detecting the chain ends.[5]

[5] See for example V. Poirier *et al.* Pharmaceutical Polymer Analysis Using Benchtop NMR: Compendial Testing of Alcohol Ethoxylates and Beyond, October 2024.

In the specific case of PLGAs, NMR is the routine analytical technique for structural characterization in research and development stages, offering a key solution to directly measure the LA:GA ratio. Additionally, it supports sophisticated and highly detailed microstructural investigations.[6] Interestingly, both the ChP and the USP–NF monographs have chosen NMR for compendial testing of their listed PLGAs, specifically for systematically testing the LA:GA ratios. However, these monographs do not require the direct control of PLGAs' molecular weight either by GPC or NMR.[7] Instead, they rely on the aforementioned, simpler viscosity measurement for bulk control. In addition, the USP–NF monographs for PLGA use NMR for the first identification test by comparing the sample to a reference standard, while the ChP relies on a less-specific infrared-based method.

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

This paradigm is now shifting. New excipients and modalities require more advanced analytical technologies. In addition, regulatory bodies are demanding a deeper understanding of analytical procedures and their applications, e.g. with the formal implementation of Analytical Quality by Design (AQbD) (ICH[8] Q14) principles. This new, comprehensive framework encourages living and agile procedures. It calls for modern approaches and technologies, such as NMR, as detailed in the ICH Q2(R2) guidelines on "Validation of Analytical Procedures." Ongoing revisions of the USP–NF general chapters ‹761› and ‹1761› also attest to this progress.[9] The key steps forward in legislation coincide with recent improvements in Good Manufacturing Practices (GMP) compliance support for NMR spectrometers and the introduction of benchtop systems, such as the Bruker Fourier 80.

The Fourier 80 is indeed an ideal tool for QC laboratories, offering the benefits of NMR analysis through a compact, cryogen- and maintenance-free system that fully complies with pharmaceutical regulations for the implementation of compendial and custom procedures. This aspect is particularly attractive for laboratories controlling polymeric materials, as NMR plays a critical role.

This whitepaper discusses how the Fourier 80 can easily be used for the control of PLGAs, supporting QC laboratories with a modern, cost-effective alternative to high-field NMR systems while ensuring fit-for-purpose procedures. It must be mentioned that the future USP–NF monograph listed in Table 1 requires a minimum 300 MHz NMR field, i.e., a high-field spectrometer. However, this document showcases that the procedure can also be successfully implemented on a benchtop system, yielding virtually identical results for the LA:GA ratio determination. Therefore, the Fourier 80 constitutes an attractive system for alternative procedures.

The document then discusses how the use of this instrument can be extended to other polyesters, through the concept of platform procedures introduced by AQbD for robust yet flexible modern analytical procedures. Additional uses of the Fourier 80 will also be illustrated for polymer. These insights help illustrate the unique capabilities of NMR and the Fourier 80 for streamlined polymer control.

[6] See for example Beck-Broichsitter *et al.* Drug Deliv. and Transl. Res. **2022**, *12*, 720.

[7] USP-PF proposed new general chapters <315> for the number-average molecular weight determination by NMR of lactide or lactic acid and glycolide or glycolide acid polymers and <316> for the molecular weight and polydispersity determination by GPC using universal calibration for lactide or lactic acid and glycolide or glycolide acid polymers, not yet official.

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

[9] As of December 2024.

PLGA control using the Benchtop Fourier 80

The compendial testing of PLGAs described in the ChP for LA:GA ratio determination (referred to as "Molar Content of Lactide and Glycolide") is strictly identical for the three materials listed in Table 2. Similarly, the LA:GA testing procedures prescribed by the USP–NF monographs (referred to as "Ratio of dl-Lactide to Glycolide in Copolymer") are identical for all 50:50 PLGAs in Table 1 . Furthermore, the ChP and USP–NF procedures are very similar. Besides the USP–NF specific requirement for a high-field system, they differ only in certain descriptions, such as the concentration of the sample, which is not relevant for this type of test.

In more detail, the compendial test simply consists of:

- Dissolving the PLGA sample that is to be tested in deuterated chloroform containing tetramethylsilane as reference for internal chemical shifts.
- Recording and processing the corresponding ^{1}H NMR spectrum for quantitative applications.
- Integrating the resonances associated to the LA and GA repeating units to calculate the effective values.

It is important to emphasize the simplicity of such an NMR test. In effect, it provides a key tool to analyze directly and non-destructively a critical structural property of the polymer, which is otherwise challenging to measure. Making the analytical process even smoother, the test descriptions for LA:GA ratio determination in the ChP and USP-NF monographs are rather short and straightforward. These refer to their corresponding NMR general chapters for most of the test. The only specific prescriptions in the monographs are the solvent to be used, the ^{1}H NMR resonances to be exploited, and the calculation of the reportable value from the relative integration results. All other considerations about the execution of NMR-based testing follow the general chapters, clearly showcasing how NMR-based procedures can help establishing simple, generic testing methods that applies to different materials. It follows that NMR is well suited to the modern concept of a platform procedure, whereby the same analytical procedure can be applied to several products without changes. In this case, various types of PLGA with significantly different properties can rely on one, single testing procedure.

A typical ^{1}H NMR spectrum resulting from this procedure and acquired with the Fourier 80 is displayed in Figure 1. A commercial RG502H PLGA reference was used, corresponding to product A of Table 1 and Table 2. The figure shows the resolution of th ɜ resonances originating from each repeating unit. This, in turn, supports the direct calculation of the LA:GA ratio.

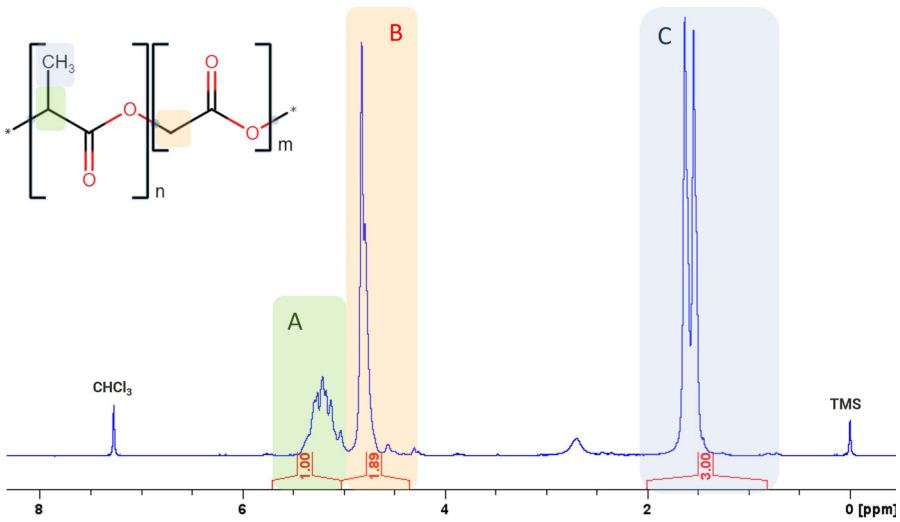

Figure 2: Example of an ^{1}H NMR spectrum of PLGA (Resomer® RG502H) recorded on a Fourier 80 in $CDCl_3$. Colored boxes indicate respective structure attributions. Integrations of the A and B areas are used to calculate the LA:GA ratio.

Indeed, under quantitative conditions, the integrals of the ^{1}H NMR resonances are directly proportional to the molar quantities of each moiety. Therefore, the mole percent ratio (mol%) of LA and GA can be easily determined using equations 1.

$$mol\%\ LA = \frac{A}{A + \frac{B}{2}} \times 100$$

$$mol\%\ GA = \frac{\frac{B}{2}}{A + \frac{B}{2}} \times 100$$

Equations 1: *Calculation of the mol% of LA and GA, adapted from USP-NF. A and B refer to the methine protons of the LA units and the methylene protons of the GA units, respectively (see Figure 1).*

The NMR integration values can also be directly converted into weight percent (weight%) values using equations 2.

$$w\%\ LA = \frac{72.1 \times A}{72.1 \times A + 58.0 \times \frac{B}{2}} \times 100$$

$$w\%\ GA = \frac{58.0 \times \frac{B}{2}}{72.1 \times A + 58.0 \times \frac{B}{2}} \times 100$$

Equations 2: Calculation of the w% of LA and GA. A and B refer to the methine protons of the LA units and the methylene protons of the GA units, respectively (see Figure 1).

Although the current ChP and USP–NF monographs only use the A and B areas to calculate the LA:GA ratio, the resonance of the methyl moiety of the LA units could be used too. In an alternative procedure for certain PLGA products, this could prove beneficial if the resolution between the A and B areas is limited. In such instances, equations 1 can be rewritten as equations 3.

$$nol\%\ LA = \frac{\frac{C}{3}}{\frac{C}{3} + \frac{A + B - \frac{C}{3}}{2}} \times 100$$

$$mol\%\ GA = \frac{\frac{A + B - \frac{C}{3}}{2}}{\frac{C}{3} + \frac{A + B - \frac{C}{3}}{2}} \times 100$$

Equations 3: Calculation of the mol% LA and GA using the C area. A+B refers to the total area consisting of the methine protons of the LA units plus the methylene protons of the GA units. C corresponds to the methyl area of the LA units (see Figure 1).

These equations are valid only if the NMR data are acquired under quantitative conditions, as specified in the compendial methods. This prerequisite can be easily met, since all variables for accurate NMR-based quantification are well understood and known.

While a comprehensive discussion on quantitative NMR is beyond the scope of this manuscript,[10] it is important to note that, when implementing a new ^{1}H NMR procedure, ensuring quantitative conditions essentially comes down to the adjustment of a single key parameter: the so-called recycle delay. This is the only analyte-dependent parameter in NMR, and all others can be generalized.

The recycle delay is set according to the slowest relaxing nucleus used for quantification. It is determined using a dedicated NMR experiment, called inversion-recovery (see Figure 3), which measures all longitudinal relaxation times (T_1). This makes it possible to calculate the appropriate recycle delay. This experiment is available in the default library of the Fourier 80 and can be automated to streamline operations. This experiment needs to be performed only once during the initial method verification process, as per USP–NF ‹1226›.

[10] Detailed discussion and guidance can be found in the current draft of USP-NF <761> and <1761>, for example.

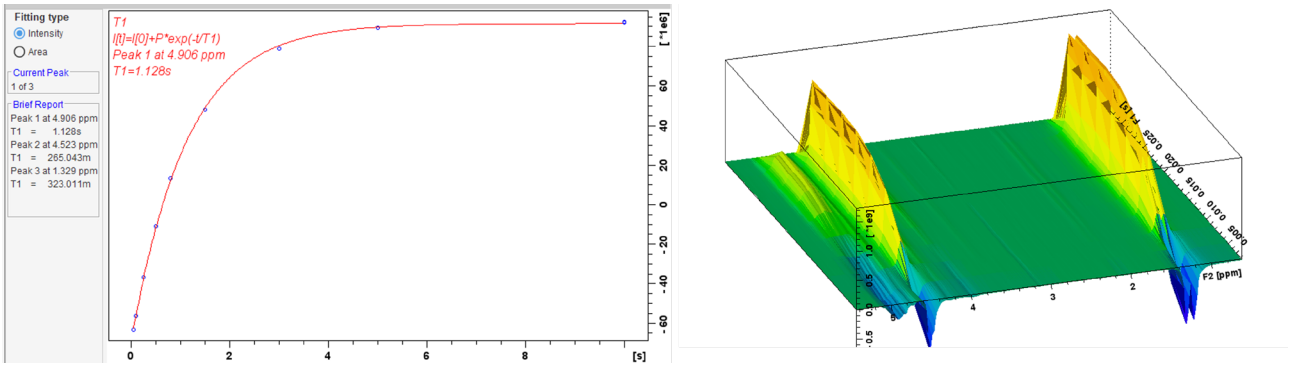

Figure 3: Example of a T_1 measurement through an inversion-recovery experiment using the Fourier 80 on a RG502 sample in $CDCl_3$. The screenshot on the right illustrates the automated calculation of the T_1 for each type of proton in the polymeric structure, supporting the direct identification of the longest value (1.1 s here). The image on the left is a 3D representation of the resulting NMR spectral data. By setting a recycle delay of at least 5x times, it is possible to ensure suitable conditions for quantitative analysis.

As mentioned in the previous sections, the USP–NF or ChP monographs do not prescribe specific NMR settings. Instead, they refer to their general NMR chapters. In addition to setting quantitative conditions by specifying an appropriate recycle delay, laboratories can thus adjust other parameters, based on their specific expertise and system capabilities.

For example, the ^{13}C decoupling capability of the Fourier 80 can be advantageous, as it can be used to suppress the coupling between protons and the NMR-active ^{13}C isotope, which accounts for 1.1% of the total carbons present. This $^{1}H\{^{13}C\}$ acquisition scheme merges the side resonances with the main resonances, producing a cleaner baseline (Figure 4). Moreover, this option is beneficial for high-accuracy quantitative NMR applications, as it can help minimize interferences, and is generally considered good practice in modern quantitative NMR.

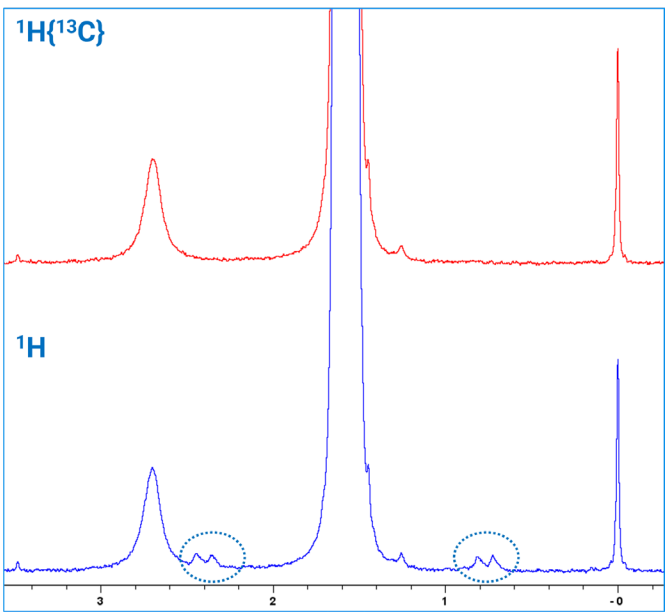

Figure 4: Example of zoomed in ^{1}H NMR spectra of PLGA (Resomer® RG502H) recorded on a Fourier 80 in $CDCl_3$ with (top) and without ^{13}C decoupling (bottom). Dotted circles indicate the detectable ^{1}H-^{13}C side resonances, suppressed when adopting a $^{1}H\{^{13}C\}$ sequence. While the zoom focuses on the resonance associated with the methyl group of the LA units, the same principle applies to all other C-bound proton resonances of PLGA.

Table 3 summarizes the results obtained on a PLGA Resomer® RG502H sample collected using two different Fourier 80 instruments, with three different concentrations, and with systematic replicates to assess precision. In addition, ^{13}C decoupling was also systematically assessed. To provide a clearer view, only mol% LA is reported, as mol% GA or the corresponding w% can be directly deduced.

Instrument	Sample	Concentration (mg/mL)	Replicates	^{1}H		$^{1}H\{^{13}C\}$	
				Result (mol% LA)	%RSD	Result (mol% LA)	%RSD
1	1	37	6	51.4	0.1	51.4	0.1
2	1	37	3	51.4	0.2	51.4	0.1
2	2	22	3	51.6	0.1	51.6	0.1
2	3	52	3	51.2	0.1	51.3	0.0
			Total average	51.4	0.3	51.4	0.3

Table 3: mol% LA determined on a batch of PLGA Resomer® RG502H using the Fourier 80 under various conditions and systems. The averages of all replicates are reported. All results were obtained in full automation mode (acquisition and processing).

All the results presented were obtained through fully automated acquisition and processing (see Figure 5), eliminating any possible bias from manual processing. Thus, they can be considered as representative of routine, automated measurements, which is highly desirable for QC.

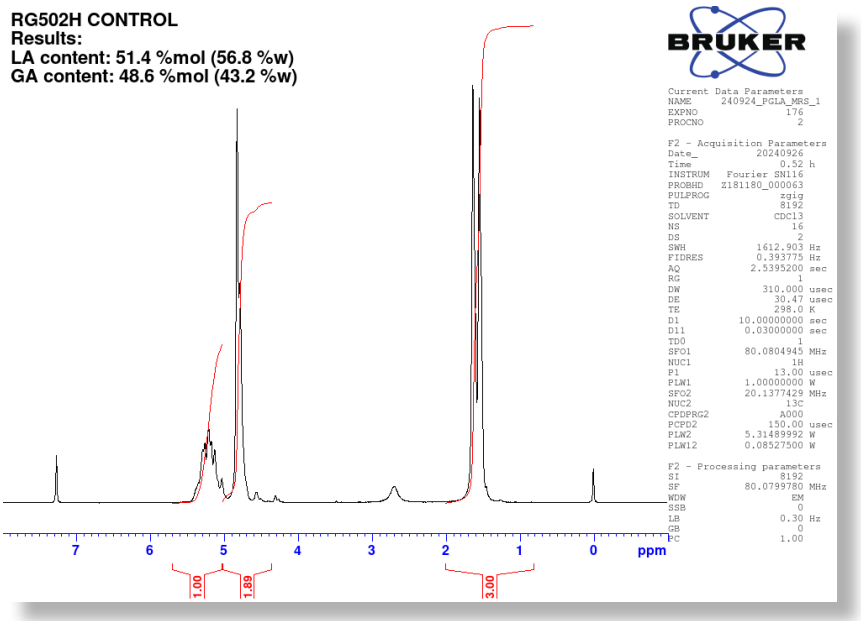

Figure 5: Example of a report obtained through the automated acquisition and processing of PLGA (Resomer® RG502H) using the Fourier 80.

To offer a clear comparison between benchtop NMR and high-field systems as well as to gain insights on accuracy, the studied batch was also tested on a Bruker AVIIIHD 400 MHz system, which resulted in a reference value of 50.8 mol% LA. These tests demonstrates several key features of NMR in general and, more specifically, the Fourier 80 for PLGA control. More precisely:

- Virtually identical results were obtained across different systems, with the values obtained with the Fourier 80 being extremely close to those obtained with higher field NMR. This confirms that the lower resolution and sensitivity of the Fourier 80 do not compromise the performance and quality of results required for PLGA testing.
- Excellent repeatability and intermediate precision were achieved, with relative standard deviations systematically and consistently below 0.5%. This attests to the ability of the Fourier 80 to offer high precision in full automation mode.
- General robustness of NMR was evidenced. Even though the compendial tests specify the concentrations to be used, these results highlight that these are not critical, and that a much broader concentration range can successfully be used. Similarly, the acquisition scheme (e.g., with or without carbon decoupling) does not affect the end results. This is to be expected, as in quantitative NMR settings, the experimental conditions should not influence the absolute outcome.

The overall robustness provided by the Fourier 80 for PLGA analysis can also be qualitatively observed by overlaying the spectra recorded during this study. As illustrated in Figure 6 using the spectra recorded with the $^{1}H\{^{13}C\}$ acquisition scheme and normalized in height, barely any differences can be observed from spectrum to spectrum. The only exception is the resonance originating from the exchangeable protons in the 3.2–2.2 ppm range, which include residual water. This is expected as this resonance will shift depending on, among other factors, the actual concentration. Remarkably, the qualitatively matching overlay applies to replicates of the same sample as well as various other preparations and systems tested over dozens of hours, attesting to the extreme stability and robustness provided by the Fourier 80.

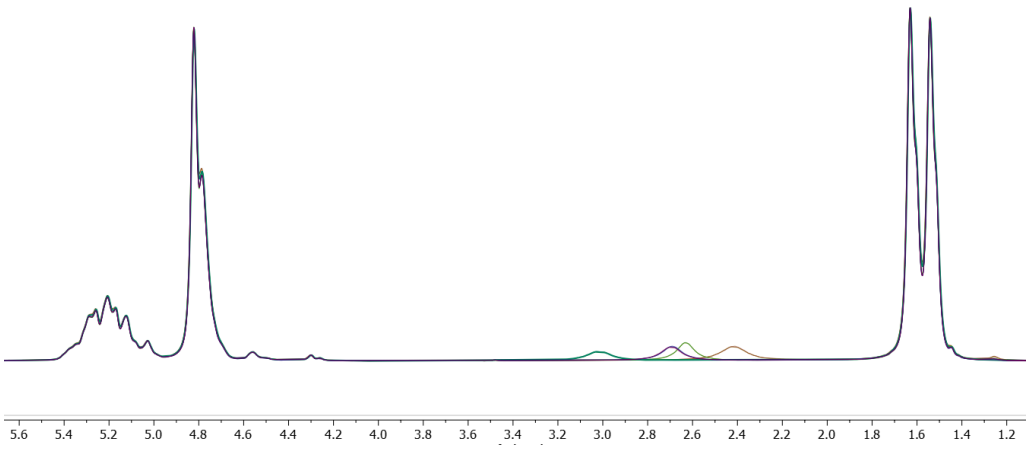

Figure 6: Overlay normalized in height for 15 ^{1}H NMR spectra of PLGA (Resomer® RG502H) recorded on Fourier 80 in $CDCl_3$ for the study reported in Table 3 with a $^{1}H\{^{13}C\}$ acquisition scheme.

As previously mentioned, this analytical procedure is virtually identical for all PLGAs listed in the compendia and can be applied to any PLGA. To illustrate the versatility of the solution, two other PLGA references were tested using the same procedure, this time with a 75:25 LA:GA ratio and either ester (Resomer® RG755S) or an acid termini (Sigma-Aldrich ref. 806358, referred to as PLGA-75A hereafter). Spectra obtained for such samples using the same procedure with the Fourier 80 are shown in Figure 7.

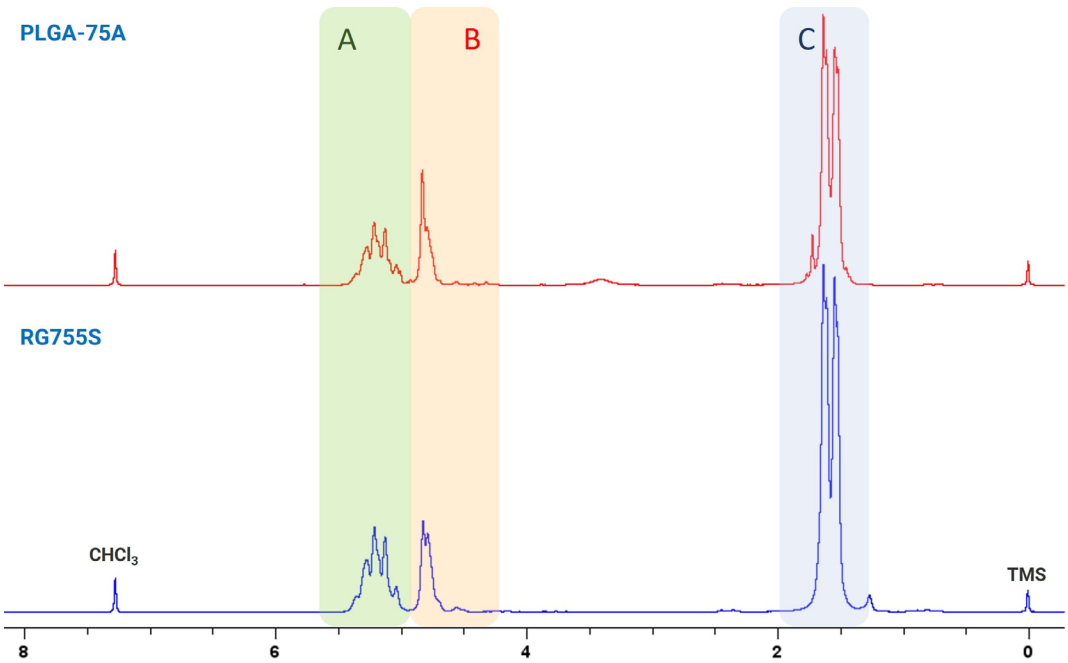

Figure 7: Example of ^{1}H NMR spectra of PLGA with 75:25 LA:GA ratio recorded on a Fourier 80 in CDCl3. Top spectrum was obtained from testing Sigma-Aldrich ref. 806358 and bottom spectrum from testing Resomer® RG755S. Colored boxes indicate respective structure attributions, as seen in Figure 2.

By comparing the spectra from Figure 2 and Figure 7, it is possible to observe how NMR can be used for polymer identification. In effect, through the resulting spectra, it is possible to confirm that the polymer under investigation is PLGA, as it matches the resonances in both chemical shift and shape, (as leveraged for the identification tests in the USP–NF monograph). The only major difference is the change in the relative ratio between the resonances associated with the LA units (A and C) and those of the GA units (B). A careful inspection of the spectra further reveals subtle changes in the shape of the PLGA resonances based on the batch. These differences are associated with minor changes in the polymer microstructures that depends on chain length, dispersity, termini, and the manufacturing processes. However, such differences do not preclude the determination of the LA:GA ratio, which can be obtained by applying the same procedure and adapting the limits of the integration when required.

A summary of the results from the investigated batches are presented in Table 4 for RG755S and Table 5 for PLGA-75A. All data were obtained through fully automated acquisition and processing. Similarly to the previous study, the mol% LA was also determined at 400 MHz for comparison, leading to values of 75.6 and 73.1, respectively.

Instrument	Sample	Concentration (mg/mL)	Replicates	^{1}H		$^{1}H\{^{13}C\}$	
				Result (mol% LA)	%RSD	Result (mol% LA)	%RSD
1	1	37	6	76.4	0.1	76.4	0.1
2	1	37	3	76.2	0.1	76.4	0.2
2	2	26	3	76.1	0.1	76.3	0.1
2	3	49	3	76.4	0.1	76.4	0.1
			Total average	76.3	0.2	76.4	0.1

Table 4: mol% LA determined on a batch of Resomer® RG755S using the Fourier 80 under various conditions and systems. Averages of all replicates are reported. All results were obtained in full automation mode (acquisition and processing).

Instrument	Sample	Concentration (mg/mL)	Replicates	^{1}H		$^{1}H\{^{13}C\}$	
				Result (mol% LA)	%RSD	Result (mol% LA)	%RSD
1	1	25	3	73.8	0.0	73.6	0.1
1	2	51	3	73.9	0.0	73.8	0.1
1	3	49	3	73.7	0.1	73.7	0.1
			Total average	73.8	0.2	73.7	0.2

Table 5: mol% LA determined on a batch of PLGA-75A (Sigma reference 806358) using the Fourier 80 under various conditions. Average of all replicates are reported. All results were obtained in full automation mode (acquisition and processing).

These additional figures further support the conclusions drawn from the study on the RG502H product. Namely, the excellent repeatability, intermediate precision, and general robustness of the Fourier 80 benchtop NMR, with consistent results obtained under different experimental conditions. Noteworthily, results obtained on the Fourier 80 are highly comparable with those obtained at higher field, demonstrating the capability of the Fourier 80 to perform this test like any other NMR technology. In a broader context, this fact also demonstrates the universal nature of NMR analytics, which can accurately determine absolute characteristics independently of the specific laboratory settings.

Extension to other polyesters – Example of poly(lactide-co-caprolactone)

The scope of application of the analytical method discussed in the previous section extends far beyond the PLGAs listed in the Compendia and other PLGAs. In fact, it is a general method that can be virtually applied to the control of any copolymers, provided that the following conditions are met:

- The polymer can be dissolved in a solvent.
- At least one ^{1}H resonance per type of unit can be specifically detected and integrated.

As long as these two criteria are fulfilled, determining the relative ratio of the blocks in the copolymers is a straightforward application of the same general procedure. It only requires adjusting the recycle delay during acquisition, setting limits of the integration areas during processing, and expressing the results in a suitable manner. With minimal verification, the procedure can therefore be applied to virtually any soluble copolymer, offering significant flexibility.

In this regard, the present procedure is highly comparable to those described for the compendial testing of Poloxamers[11] or Betadex.[12] These two classes of polymeric excipients differ from PLGA in terms of material characteristics and applications. Nonetheless, the NMR compendial methods to analyze the distribution of monomeric units are virtually identical and could be managed with a global platform approach, drastically simplifying analytical procedure life-cycle management.

To further demonstrate this versatility, the same procedure was applied to poly(lactide-co-caprolactones) (PLCs), another class of biodegradable polyesters, where ε-caprolactone (CL) is copolymerized with LA instead of GA. Figure 8 shows the generic structure of PLC and presents a typical example of the ^{1}H spectrum of a commercial PLC copolymer (with theoretical LA molar ratio of 86%) recorded on a Fourier 80.

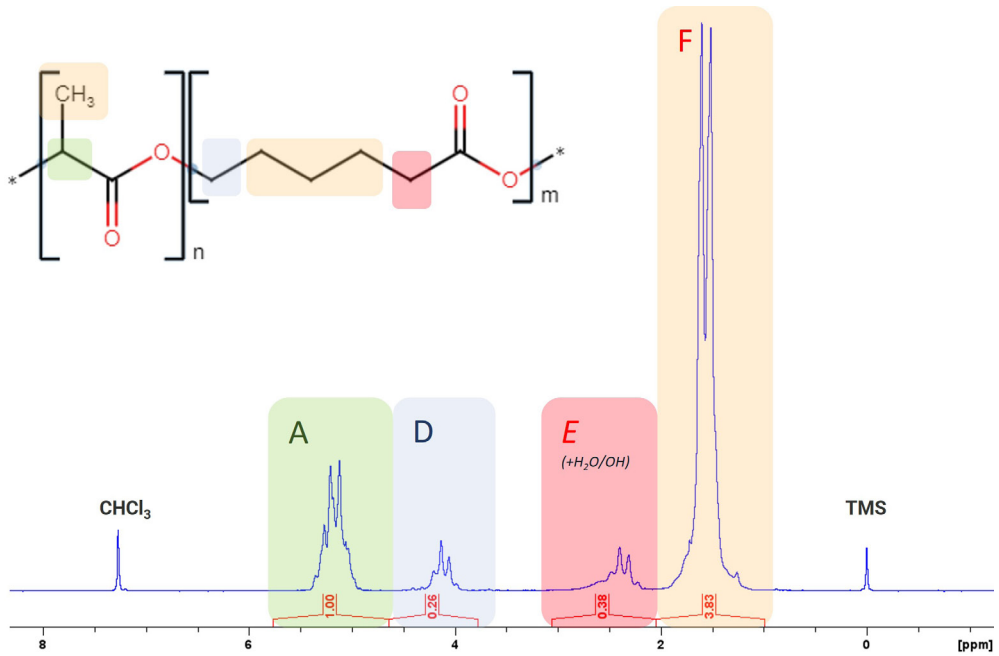

Figure 8: Example of an ^{1}H NMR spectrum of PLC (Sigma-Aldrich ref. 457647) recorded on a Fourier 80 in $CDCl_3$. Colored boxes indicate respective structure attributions. Residual water and exchangeable protons cause interference in area E.

[11] See for example V. Poirier *et al.* Benchtop NMR as a Versatile Tool for Quality Control: Example of Poloxamer Compendial Testing, June 2024

[12] See for example 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

Comparison with the previously discussed ^{1}H spectra of PLGA reveals significant changes in the ^{1}H resonance patterns, highlighting the ability of NMR to directly provide detailed information for identification purposes. For well-characterized products such as PLC, the structural attribution of the resonances shown in Figure 8 can be directly performed by analytical experts or referenced from the literature. Nonetheless NMR structural characterization using the same system can be used for attribution, as demonstrated with PLGA in the next section.

Since the resonances specific to the LA and caprolactone (CL) repeating units are directly accessible for integration (respectively the A and D areas of Figure 8), the procedure discussed for PLGA can be applied. It is only necessary to adapt the integration area limits. As area D accounts for two protons of the CL unit. Equations 1 should then be simply modified by substituting the GA value for area B value with the CL value for area D value, resulting in equations 4. Similarly, the w% calculation in equations 2 should be modified by substituting the molecular weight of GA with that of CL, namely 114.1 instead of 58.0.

$$mol\%\ LA = \frac{A}{\left(A + \frac{D}{2}\right)} \times 100$$

$$mol\%\ CL = \frac{\frac{D}{2}}{\left(A + \frac{D}{2}\right)} \times 100$$

Equations 4: Calculation of the mol% LA and CL in PLC. A and D refer to the methine protons of the LA units and the O-bounded methylene protons of the CA units, respectively (see Figure 1).

Just like in the PLGA studies, the excellent precision and robustness of the procedure are illustrated through the systematic and automated analysis of preparation and acquisition replicates using different systems and conditions. Results are summarized in Table 4.

Instrument	Sample	Concentration (mg/mL)	Replicates	^{1}H		$^{1}H\{^{13}C\}$	
				Result (mol% LA)	%RSD	Result (mol% LA)	%RSD
1	1	37	6	88.2	0.0	88.3	0.0
2	1	37	3	88.1	0.0	88.4	0.0
2	2	26	3	88.2	0.1	88.4	0.0
2	3	45	3	88.3	0.0	88.5	0.0
			Total average	88.2	0.1	88.4	0.1

Table 5: %mol LA determined on a batch of PCL (Sigma-Aldrich ref. 457647) using the Fourier 80 under various conditions and systems. Averages of all replicates are reported. All results were obtained in full automation mode (acquisition and processing).

Comparing these results to the value determined at 400 MHz (mol% LA 88.1) further underscores the accuracy retained when performing such analytical tests on the Fourier 80. This indicates that the instrument is well suited for determining the block composition in PLGAs, PLCs and, more generally, polyesters, offering a single, unified platform approach.

Beyond routine testing, use of the Fourier 80 for structural characterization / 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 an extensive range of NMR experiments originally developed for high-field systems. The Fourier 80 has a global topology that is 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). As a result, one-dimensional and multi-dimensional experiments can be performed using the same experiment libraries and just as they would with high-field systems. Consequently, users can easily shift between systems to leverage the solutions best suited to address specific needs.

To exemplify these capabilities, Figure 9 presents typical NMR experiments used for structural characterization. These include multidimensional Heteronuclear Single Quantum Coherence (HSQC) and Heteronuclear Multiple Bond Correlation (HMBC) experiments, complemented by a ^{13}C spectrum with proton decoupling and the corresponding structural attribution.

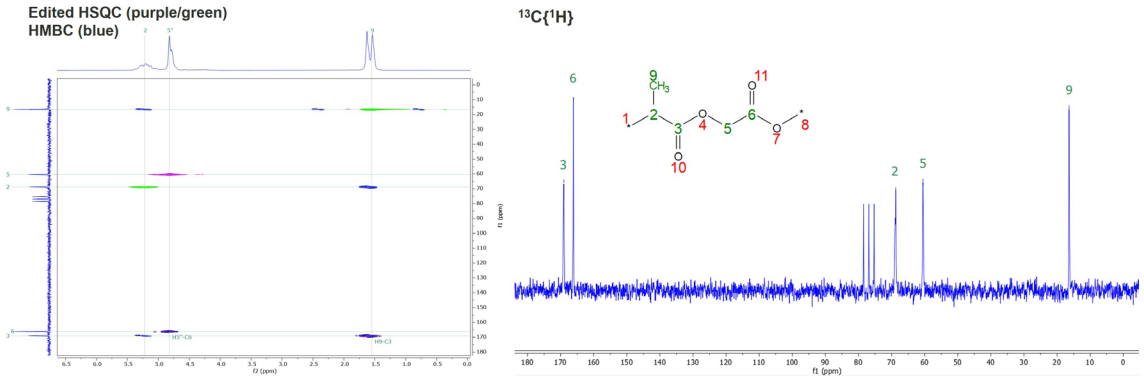

Figure 9: Example of possible structural characterization via NMR using the Fourier 80 with a sample of PLGA RG502H (250 g/L in $CDCl_3$): overlay of edited-HSQC and HMBC spectra (left) as well as $^{13}C\{^1H\}$ spectrum (right) with attribution of the carbon resonances.

While a detailed discussion of NMR experiment selection and interpretation for structural analysis is beyond the scope of this manuscript, these investigations illustrate the capabilities and versatility of the Fourier 80 to perform a variety of NMR experiments. These range from robust routine testing to more demanding studies that combine multiple experiments for in-depth characterization.

The complexity of PLGAs requires the resolution and sensitivity of high-field NMR systems for detailed microstructural investigation and fine characterization of the specific monomer sequence distribution along the polymer chain.[6] Nonetheless, the Fourier 80 can offer a simplified and cost-effective access to a number of bulk characteristics for first-level investigations, helping laboratories reserve the more demanding high-end NMR systems solely for challenging studies.

Conclusion

NMR is a cornerstone analytical technique for the characterization of organic polymers, capable of providing detailed qualitative and quantitative information otherwise impossible to obtain. In this document, we demonstrated the critical role of NMR spectroscopy in the structural characterization and quality control of PLGA polymers. The paper showcases how the benchtop Bruker Fourier 80 NMR system can be a valuable tool for routine QC of PLGA composition, offering an attractive, cost-effective alternative to high-field NMR systems. In effect, it can be successfully used for compendial testing according to the ChP or as an alternative procedure for the USP–NF.

The versatility of the Fourier 80 extends beyond PLGAs, demonstrating its potential for broader applications in polymer analysis, provided a few criteria are met. These opportunities clearly highlight the advantages of NMR and the Fourier 80 for QC, as they support recent platform procedure concepts, simplifying and streamlining analytical procedure management. As regulatory requirements evolve, the adoption of advanced analytical techniques like NMR will be pivotal in meeting the demands for deeper understanding and control of pharmaceutical products.

The Fourier 80 delivers the inherent benefits of NMR within a compact, cryogen-free system that requires virtually no maintenance. With built-in GMP support for regulatory compliance and full automation capabilities, it offers an ideal, cost-effective solution for laboratories interested in integrating NMR and maximize the benefits of the analytical method as a modern technology for QC.

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