PHARMA

Benchtop NMR as a Versatile Tool for Quality Control: Example of Poloxamer Compendial Testing

Michal Stricker, Barbara Czarniecki, Clemens Anklin, Fabrice Moriaud, Victor Beaumont, Anna Codina and Valentin Poirier
June 2024

Innovation with Integrity

Poloxamers are essential nonionic surfactants in pharmaceutical formulations. Their composition significantly influences their functional properties, necessitating NMR analysis for regulatory compliance as outlined in their USP-NF and Ph. Eur. monographs. The Bruker Fourier 80 benchtop NMR spectrometer is an ideal solution for such testing: it meets all technical requirements of the monographs, while bringing NMR capabilities in a compact, cryogen-free system, well-suited for quality control laboratories. Based on years of experience, Bruker GxP kits support achieving the required full compliance with the regulatory requirements without compromising ease of use and automation features.

Poloxamers are a class of triblock copolymers, consisting of a central polypropylene oxide (PPO) block linked to two polyethylene oxide (PEO) chains (Figure 1). They are archetypical examples of nonionic surfactants, the PPO core being hydrophobic while the PEO chains provide the hydrophilicity. Since their first patent by BASF in the 1950s as detergents, they have garnered significant and growing attention in pharmaceutical formulations.[1] Originally introduced under the trade name Pluronic®,[2] these synthetic polymers gained prominence for their ability to stabilize pharmaceutical formulations by reducing surface and interfacial tensions. This is crucial in preventing protein aggregation and denaturation. Among the various marketed products, Poloxamer 188 and Poloxamer 407 have been particularly noted for their efficacy in drug solubilization and stabilization, demonstrating safe and effective use across multiple drug formulations.[3]

[1] Kabanov et al., Journal of Controlled Release, **2002**, 82, 189-21

[2] Several trade names have since emerged such as Lutrol®, Kolliphor®, Antarox®, and Synperonics®.

[3] Thomas et al. Acta Biomaterialia, **2020**, 110, 37-67; Muniz et al. Biomaterials Advances, **2023**, 151, 213484

Poloxamers are particularly important in protein-based therapeutics or biologics, which are prone to instability and aggregation. In this context, poloxamers not only enhance the physical stability of these sensitive molecules but also improve their bioavailability and therapeutic efficacy.[4] Comparatively, poloxamers present advantages over traditional pharmaceutical surfactants like polysorbates, which have been associated with issues such as degradation and particulate formation under stress conditions.[5] Thus poloxamers are increasingly preferred in formulations that are susceptible to oxidative stress or require stringent storage conditions.[3]

Pharmacopeias have acknowledged the importance of poloxamers by including them in the excipient/reagent categories for many years. Both the major United States Pharmacopeia-National Formulary (USP-NF) and the European Pharmacopoeia (Ph. Eur.) have a monograph[6] describing a range of poloxamers, as illustrated in Table 1, while the Chinese Pharmacopeia focuses, for example on the most common poloxamers 188 and 407, with identical specifications. Yet it should be noted that the references listed in these monographs represent only a fraction of the various poloxamers commercially available.

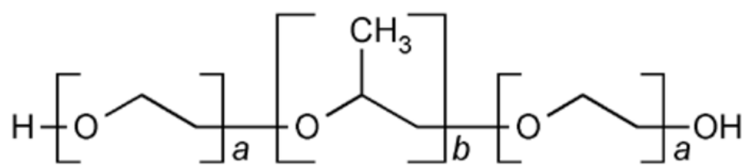

Figure 1 Schematic structure of poloxamer as reported in USP-NF.

Poloxamer	Physical Form	Average Molecular Weight	Weight % Oxyethylene	a	b
124	Liquid	2090 to 2360	46.7 ± 1.9	12	20
188	Solid	7680 to 9510	81.8 ± 1.9	80	27
237	Solid	6840 to 8830	72.4 ± 1.9	64	37
338	Solid	12700 to 17400	83.1 ± 1.7	141	44
407	Solid	9840 to 14600	73.2 ± 1.7	101	56

Table 1 Poloxamer specifications of the USP-NF monograph. Ph. Eur. monograph specifications are identical except for the a and b values indicated as ranges.

Since the self-assembly and thermogelling properties of poloxamers are intrinsically connected to the length and ratio of each block,[7] control of these characteristics is crucial to ensure the conformity of a given batch of product for pharmaceutical use. This is overall achieved in both the USP-NF and Ph. Eur. monographs by a combination of two methods:[8]

a. A dedicated, titration-based methodology to measure the overall average molecular weight.

b. A Nuclear Magnetic Resonance (NMR) method to determine the weight percent of oxyethylene (also known as PEO content or EO/PO ratio), which directly correlates to the ratio between the a and b blocks.

[4] Lamprecht et al. Pharmaceutics **2022**, 14, 2575

[5] Wang et al. J. Pharm. Sci. **2019**, 3, 1264-1271; Grapentin et al. J. Pharm. Sci., **2020**, 109, 2393-2404

[6] Numbered 1464 in Ph. Eur.

[7] The "xyz" number used in monographs indicate the actual ratio and length. The digits xy multiplied by a factor of 100 corresponds to the approximate molecular mass of the oxypropylene center and z multiplied by 10 describes the oxyethylene content in percentage. Not all trade names however use the same nomenclature.

[8] Ph. Eur. also uses an additional IR-based method for identification.

NMR spectroscopy is a very well-established method for structural characterization. In the case of polymers, NMR is the only analytical technique able to provide detailed characterization of their composition and probe their microstructure, with the significant advantages of being inherently quantitative and non-destructive. Determining the ratio of repetitive units in block copolymers is thus straightforward with NMR, while practically impossible with any other techniques, or at least without very significant effort such as complete denaturation of the product. Average chain length (and thus average molecular weight in number) can also be generally determined for well-defined polymeric structures, exploiting the chain-end resonances. Modern NMR approaches such as diffusion-ordered spectroscopy (DOSY) can also give direct access to information about the statistical distribution similarly to size exclusion chromatography (SEC).

Pharmacopeia methodologies, however, intend to rely on the most "accessible" procedure in regards of equipment and expertise. For a long time, NMR was perceived as a technique to avoid for compendial procedures due to the cost of the systems and the level of expertise required. Although NMR has been rapidly established as one of the fundamental analytical techniques in academia, its availability in the private sector was much more limited, and GMP-compliant systems were even rarer. This paradigm is now rapidly changing. Spectrometers are simpler to use and can be operated on a routine basis by non-experts, because of significant improvements in user interfaces, fully automated procedures, and simplified maintenance operations. The recent progress in GMP compliance of NMR spectrometers and the introduction of benchtop systems have also further accelerated the process, as evidenced by the recent introduction of NMR in the ICHQ2(R2) and the ongoing revision of USP <761> and <1761>.[9]

In this historical context, the procedure for the control of the overall average molecular weight in the poloxamer monograph is a typical example: a lengthy and tedious chemical titration methodology was, at the time, chosen over NMR or SEC as it only requires a few chemicals and glassware. If this choice could be revisited nowadays, no alternative was found from the beginning for the weight percent oxyethylene determination, and NMR was indeed introduced as the compendial tool. As such, the historical poloxamer monographs can be seen as a precursor for the introduction of modern techniques in pharmacopeias.

The weight percent oxyethylene testing of poloxamer by NMR is straightforward: it consists of a simple sample preparation (dissolution in a deuterated solvent), spectrum acquisition and data analysis. Details of the analytical procedures described in the USP-NF and the Ph. Eur. are thus very similar. The only significant differences are that the Ph. Eur. only indicates $CDCl_3$ as the solvent while the USP-NF indicates both D_2O and $CDCl_3$ as possibilities but requires the presence of a chemical shift standard (DDS or TMS), and more surprisingly requests the addition of one drop of D_2O if $CDCl_3$ is used. This latter instruction has been probably ignored by many laboratories, but formal justification should be provided in the local standard operating procedure. In both cases, determination of the weight percent oxyethylene is directly accessible using the ratio of integrals from the ^1H NMR spectrum. NMR being an absolute method, the areas of the resonances are indeed directly proportional to the number of associated nuclei and can be leveraged for molar ratio determination. In the present case, the area A_2 (3.8-3.2 ppm) accounts for the two repetitive units of the block copolymer (signal from CH_2/CH of the oxypropylene and oxyethylene units) while the area A_1 (around 1.08 ppm) is specific to the CH_3 group the oxypropylene unit (see Figure 2).[10] The monographs directly provide the formula (reported below) to convert these raw relative integration values into the final weight % oxyethylene results, to be compared with the specification:[11]

$$\%W_{oxyethylene} = \frac{3300 \propto}{33 \propto +58} \; where \; \propto = \frac{A_2}{A_1} - 1$$

Equation 1

[9] As of June 2024

[10] Areas are reported as specified in the monographs.

[11] See application note "Quantification of Oxyethylene in Poloxamers in Full Automation on the Fourier 80" for detailed explanation of the equation.

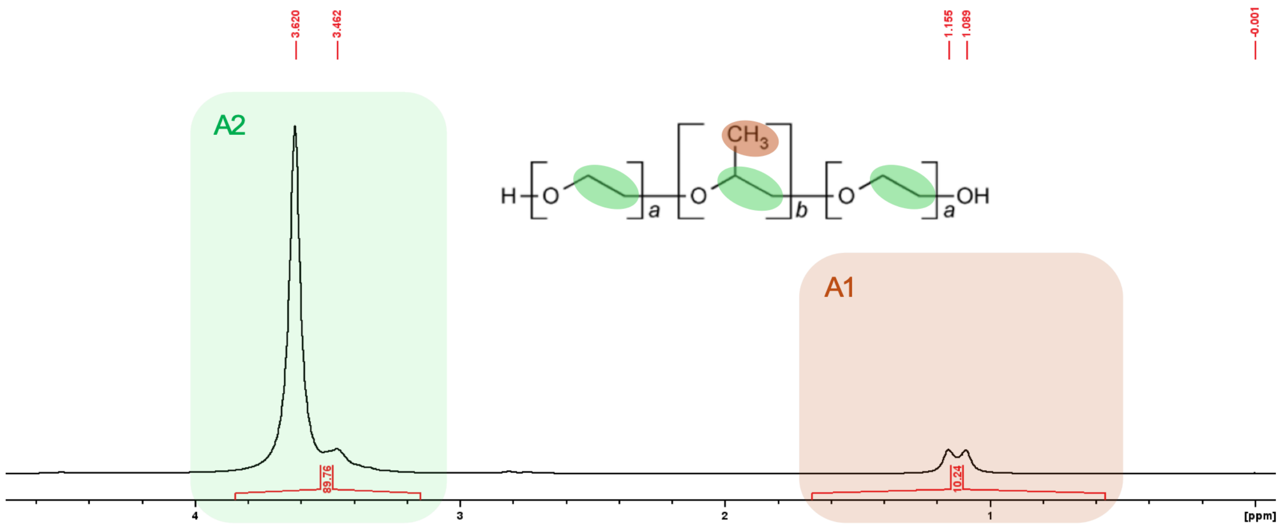

Figure 2: Example of a [1]H spectrum of a typical poloxamer 188 recorded on Fourier 80 according to USP-NF and Ph. Eur. in $CDCl_3$. A1 and A2 areas as defined in the monographs are shown in color with their structural attribution.

As demonstrated in the spectra (Figure 2), the two regions of interest, A1 and A2, are distinctly separate and the resolution of a benchtop NMR spectrometer such as the Bruker Fourier 80 significantly exceeds the requirements for individual integration of these areas. Although inherently less sensitive and resolved than high-field NMR spectrometers, the performances of the Fourier 80 spectrometer are optimally suited for such compendial testing. This system largely mitigates the challenges associated with deploying high-field NMR spectrometers in quality control laboratories while providing the simplicity and robustness required for routine testing. Table 2 and Figure 3 demonstrate the Fourier 80 successful application in verifying the conformity of various commercial poloxamers (tested according to the USP-NF and Ph. Eur. monographs in $CDCl_3$), including two specified in Table 1.

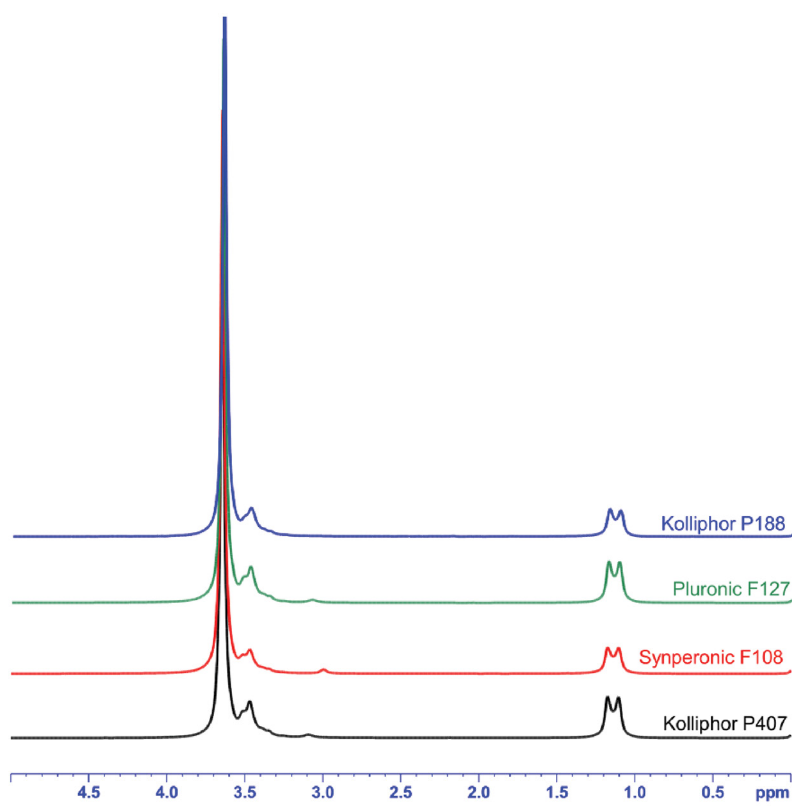

Figure 3 [1]H NMR spectra of four commercially available poloxamers recorded on Fourier 80 according to the USP-NF and Ph. Eur. monographs (zoom on the A1 and A2 regions).

Poloxamer	POE Number	Determined POE Content (%w/w)	Published POE Content (%w/w)
Kolliphor® P188	P188	80.4	80.3
Kolliphor® P407	P407	72.7	72.5
Synperonic® F108	P308	80.2	approx. 80
Pluronic® F127	P367	72.6	approx. 70

Table 2: Examples of PEO content testing using the Fourier 80 according to USP-NF and Ph. Eur. on commercial poloxamers.

To further underscore the precision of the NMR method even at lower fields, Table 3 presents the results of a repeatability study conducted on eight individual preparations of a poloxamer 188 batch. This study yielded a remarkable standard deviation of 0.2%, which is well within the most stringent USP-NF precision criteria of not more than 1% (applicable to drug substances, not excipients).

Sample	Determined POE Content (%w/w)
#1	80.1
#2	80.4
#3	80.6
#4	80.3
#5	80.5
#6	80.4
#7	80.3
#8	80.6
Average	80.4
%RSD	0.2%

Table 3: Results of a precision study for POE content testing using the Fourier 80 according to USP-NF and Ph. Eur. monographs (8 individual preparations of a poloxamer 188 batch).

Moreover, as an additional testament to the general robustness of NMR methods, retained with the Fourier 80, the determination of the weight percentage of oxyethylene was also performed at significantly lower concentrations, with dilutions of up to a factor of 60 (equivalent to 1 mg in 0.6 mL of $CDCl_3$). These concentrations were substantially below the minimum concentration stipulated by the monographs, yet the determined values (Table 4) remained within acceptable limits, only marginally exceeding the 3 σ interval based on the aforementioned repeatability results. While operating near the limit of quantification is not relevant for pure product testing, this scenario exemplifies the large operable range afforded by NMR-based analytical procedures.

Kolliphor® P188	Determined POE Content (%w/w)	SNR
4 mg	80.3	47.89
2 mg	80.1	27.55
1 mg	79.8	11.83

Table 4: Example of the impact of dilution for POE content testing using the Fourier 80 according to USP-NF and Ph. Eur. SNR is the signal to noise of the signal in the A1 region determined using a 2 ppm noise area.

Leveraging the latest advancements in hardware and software technology, the benchtop Fourier 80 NMR spectrometer presents an ideal solution for performing the compendial analyses exemplified herein. This system is well-suited for QC laboratories, offering a compact, cryogen-free setup that requires minimal maintenance and still offers the benefits and performances associated with an NMR spectrometer within a laboratory setting. Based on one of the most versatile and robust analytical techniques available, this system can be utilized for a range of NMR-based compendial tests or bespoke qualitative and quantitative procedures.[12] Additionally, it serves as a powerful tool for investigating out-of-specification and out-of-trend results from other analytical methods, enabling rapid examination of mixtures or identification of unknown products.

As concluding note, it must be emphasized that, as with all compendial procedures, execution of the weight percent oxyethylene testing to produce reportable values must adhere strictly to pharmaceutical regulations. This encompasses not only the systematic implementation of monograph requirements but also those of the associated general chapters of the relevant pharmacopeia, and more broadly, the local regulations governing pharmaceutical testing, such as cGMP. While these general frameworks are well-established within testing laboratories, the nuances associated with NMR are typically less familiar. In the case of the poloxamer monographs, both USP-NF and Ph. Eur. provide limited descriptions of NMR methods, necessitating that testing laboratories consult the corresponding general chapters for NMR[13] to ensure the procedure is suitable for the intended purpose. This process also involves defining the protocol and criteria for the procedure verification before its utilization.[14] For non-expert laboratories, the technical details contained within these general chapters may appear daunting. This potential barrier can be mitigated by the Bruker GxP Readiness Kits for Fourier 80, which facilitate complete compliance for the laboratory. Supporting documentation is supplied to assist with the initial instrument qualification, inclusive of its computerized system. Subsequently, comprehensive tools are provided to aid users in designing and maintaining continuous performance qualification. The software ensures data integrity and supports full automation for routine testing, while also offering the flexibility needed for simultaneous applications and multiple users. To illustrate this, Figure 4 demonstrates the workflow for poloxamer NMR testing using the Bruker Fourier 80 NMR spectrometer and the Bruker GxP Readiness Kit Enterprise, executed in full automation.

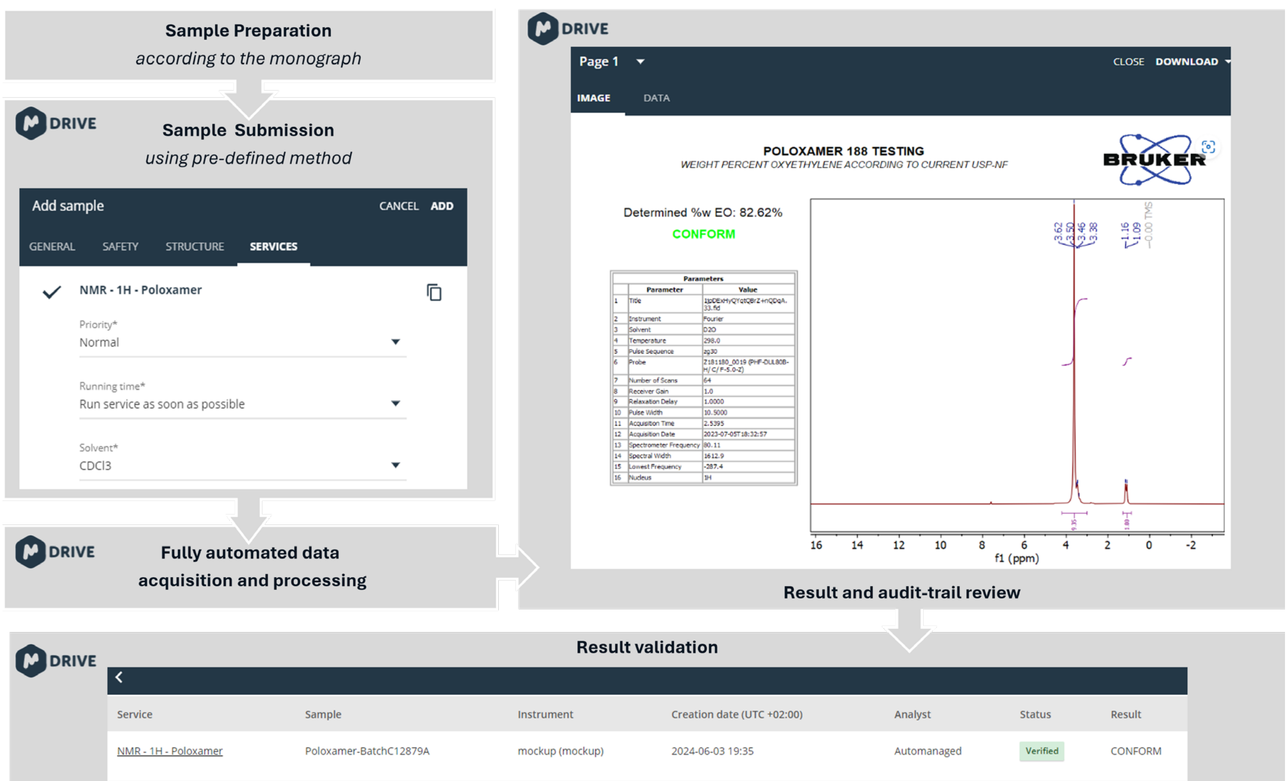

Figure 4: Workflow for fully automated poloxamer NMR testing according to USP-NF and Ph. Eur. using the Fourier 80 and the Bruker GxP Readiness Kit Enterprise. The whole analytical cycle is performed using a web interface (Mdrive). Data and audit-trails are secured on a server database, ensuring data integrity.

[12] Current drafts of <761> and <1761> as of June 2024 give a very good overview of the scope of NMR application for QC applications.

[13] <761> for USP-NF, 2.2.33 for Ph. Eur.

[14] As required by USP-NF <1226>

Bruker BioSpin
info@bruker.com

bruker.com

Benchtop NMR for Quality Control
Solution Page

Benchtop NMR for Quality Control
Download Page