NMR

Quantification of Oxyethylene in Poloxamers in Full Automation on the Fourier 80

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Innovation with Integrity

Poloxamers are synthetic block copolymers composed of a central hydrophobic oxypropylene chain connecting two hydrophilic oxyethylene ends. An attractive variety of valuable applications in fields such as drug delivery and cosmetics have been found, taking advantage of their amphiphilic and surfactant properties. The quantification of the oxyethylene content is of great interest. A poloxamer's ability to self-assemble and form a thermo-gel for instance is highly related to its composition. To quantify the oxyethylene weight percentage in poloxamers USP-NF (United States Pharmacopeia - National Formulary) suggests Nuclear Magnetic Resonance Spectroscopy. [1] This official quantification procedure described by the USP-NF was performed in an entirely automized fashion on the Fourier 80, Bruker's FT-NMR benchtop spectrometer.

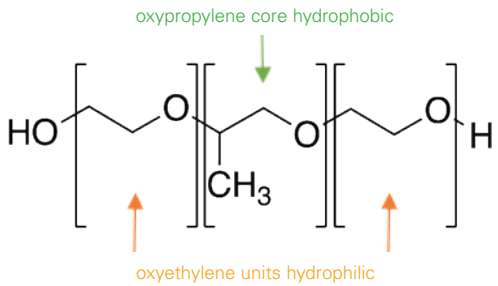

Figure 1: General structure of a poloxamer with its repeating units.

Multiple poloxamers differing in the amount of repetition units are commercially available under the trade names Kolliphor®, Pluronic® and Synperonic®. They are identified by a characteristic Poloxamer number "xyz", where 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.[1] ^{1}H 1D NMR spectra of poloxamers show two significant peak regions; one region at approximately 3.7 ppm, corresponding to the -CH_2 and -CH groups and a doublet at around 1.1 ppm resulting from the oxypropylene methyl groups.

[1]Notice, the Poloxamer number is only equivalent to the code mentioned in the trade names of Kolliphor®, not however of Pluronic® and Synperonic®.

A range of poloxamer samples (Kolliphor®, Pluronic® and Synperonic®) in different concentrations and solvents was investigated. The samples were prepared by dissolving 60 mg of the respective poloxamer in 600 µL of $CDCl_3$ with TMS as internal standard. Concentration, solvent, and internal standard were chosen according to the USP-NF. [1] USP-NF recommends D_2O as an alternative solvent with DSS as internal standard. However, the obtained oxyethylene contents of aqueous samples were less accurate due to the limited solubility of the poloxamers in D_2O.

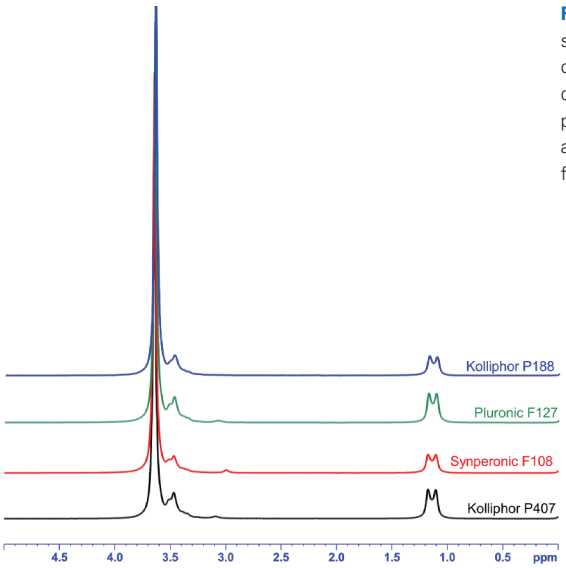

Figure 2: ^{1}H 1D NMR spectrum of four commercially available poloxamers, displaying the two distinct peak regions. The spectra are not presented in their full vertical scope.

The integral ratio of these two peak regions allows a quantitative analysis of the oxyethylene content in the poloxamer according to:

$$wt\%\ oxyethylene = \frac{3300\alpha}{33\alpha + 58}$$

where, $\alpha = \frac{I_2}{I_1} - 1$, I_1 is the area of the doublet at 1.1 ppm and I_2 is the area of the peak region at approximately 3.7 ppm.

This relation is obtained by combining the general weight percentage formula $wt\%\ oxyet = \frac{m_{oxyet}}{m_{oxyet}+m_{oxyprop}}$ with the information given by the integral of a peak and the amount of its corresponding protons:

$n_{oxyet} = \frac{I_{oxyet}}{N_{oxyet}} = \frac{m_{oxyet}}{MW_{oxyet}}$, $n_{oxyprop} = \frac{I_{oxyprop}}{N_{oxyprop}} = \frac{m_{oxyprop}}{MW_{oxyprop}}$, where $\frac{I_{oxyet}}{N_{oxyet}} = \frac{I_2 - I_1}{4}$ and $\frac{I_{oxyprop}}{N_{oxyprop}} = \frac{I_1}{3}$ and $MW_{oxyprop} = 58\ \frac{g}{mol}$ and $MW_{oxyet} = 44\ \frac{g}{mol}$.

In the following table the obtained oxyethylene contents of the four investigated poloxamers are presented in comparison to the values published by the producer and their POE number.[3]

Poloxamer	POE number	Determined POE content	Published POE content
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 1: Results of the quantification of poloxamers.

For integration, the rather new TopSpin command "sigreg" was implemented in the AU program designed for the analysis, enabling reliable automatic signal region detection in 1D ^{1}H spectra based on artificial intelligence. [3]

[3]All poloxamers measured were purchased from Merck. Kolliphor® P188 and Kolliphor® P407 come with a certificate of analysis, whereas for Synperonic® and Pluronic® the POE content can only be approximately deduced from the denomination (P308– 8 corresponds to 80%, P367 - 7 corresponds to 70%).

Error estimation

A series of measurements was performed on Kolliphor® P188 presenting the error of the method. The quantification experiment was done on 8 equivalent, but individually prepared samples (60 mg Kolliphor® P188 in $CDCl_3$) repeatedly.

Average of the determined POE content	Standard deviation
80.4%	0.2%

Table 2: Error estimation of the method.

With respect to the standard deviation of 0.2% the method can be described to be reliable and reproducible.

To get an idea of the capabilities and limitations of the Fourier 80 spectrometer, samples with low concentrations of Kolliphor® P188 in $CDCl_3$ were analyzed. Even at low concentrations of less than 1 mM (1 mg Kolliphor® P188 in 600 µL of $CDCl_3$ corresponds to approx. 0.2 mM) sensible quantification results were obtained, as presented in the following table.

Kolliphor® P188	Determined POE content	SNR
4 mg	80.3%	47.89
2 mg	80.1%	27.55
1 mg	79.8%	11.83

Table 3: Results of the automized quantification of poloxamers. Spectra processed without line broadening.

The lower the concentration, the lower the signal to noise ratio. The relative standard deviation is inversely proportional to the signal to noise ratio. [2] Consequently, the determined POE content is less accurate at low concentrations, which is in accordance with the measurements presented in table 3. This increased deviation is due to errors occurring in the integration. The measurements were done with 16 scans. It should be stated that higher signal to noise ratios can be achieved, even at low concentrations, if time is available, by increasing the number of scans.

Automation

All measurements can be done in full automation, making use of a processing script, which returns the determined oxyethylene content of the sample without any further actions needed on behalf of the user. For this automated quantification GoScan or IconNMR may be used.

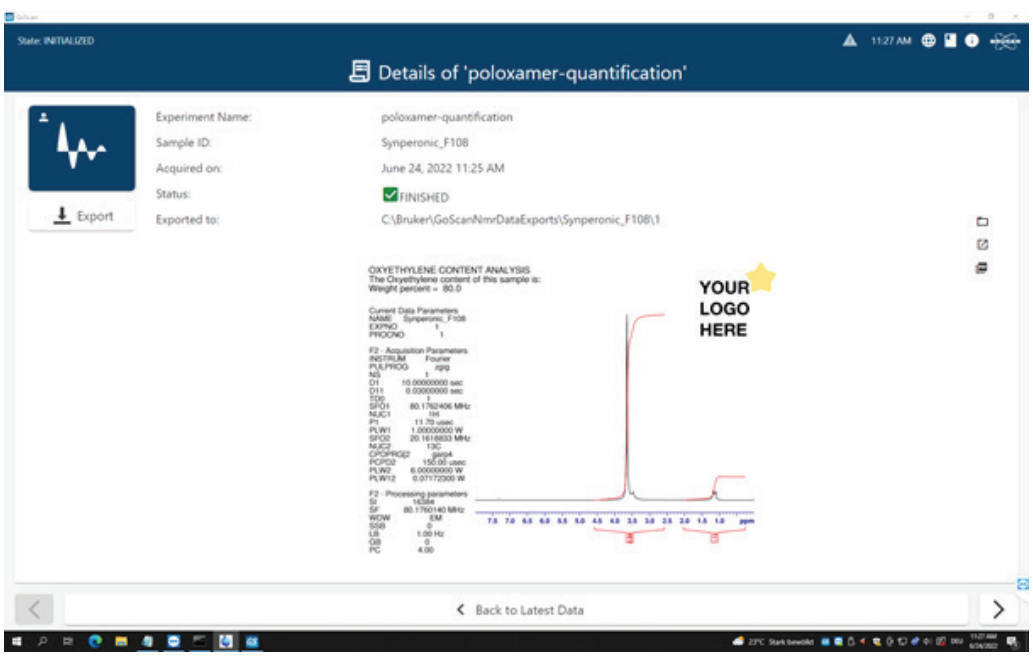

Figure 3: Outcome of the quantification done with the automation software GoScan.

Conclusion

The Fourier 80 allows for an accurate automized quantitative analysis of oxyethylene in poloxamers. Push button solutions, such as the one presented show, how accessible relevant applications of NMR spectroscopy can be made by taking advantage of a benchtop spectrometer - performing without the need of a specialist.

References:

1. United States Pharmacopeia (2022). NF Monographs, Poloxamer. USP-NF. Rockville, MD: United States Pharmacopeia. DOI: https://doi.org/10.31003/USPNF_M66210_05_01
2. https://enfsi.eu/wp-content/uploads/2017/06/qNMR-Guideline_version001.pdf
3. https://www.bruker.com/en/products-and-solutions/mr/nmr-software/topspin/_jcr_content/root/sections/more_information/sectionpar/linklist/contentpar-1/calltoaction.download-asset.pdf/links/item0/T181072_Automatic%20Signal%20Region%20Detection.pdf

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