Deconvoluting the complexity of Polysorbate 20 by direct MALDI-MS analysis using neofleX benchtop MALDI-TOF

neofleX™ benchtop MALDI-TOF provides unique capabilities for fast-turnaround in-depth characterization of complex polymer mixtures such as Polysorbate 20 accelerating macromolecular research and allowing for faster decision-making in the development of new functional polymer materials.

Abstract

Polysorbates are nonionic surfactants widely used in pharmaceutical, cosmetic, and food applications. This causes an increasing demand for analytical methods enabling time-efficient in-depth characterization of polysorbates for the purpose of quality control, stability assessment, and regulatory compliance.

In this study a direct MS method for simple and fast, though comprehensive analysis of polysorbates using neofleX benchtop MALDI-TOF is described. The method benefits from neofleX resolving power (up to R = 30,000) allowing for detection of a large variety of structurally different oligomer series, including isobaric species, in complex polymer mixtures such as Polysorbate 20.

In combination with Polymerix software for seamless data analysis, neofleX benchtop MALDI-TOF provides a rapid-turnaround workflow solution accelerating macromolecular research and allowing for faster decision-making in the development of new functional polymer materials.

Keywords:
neofleX, MALDI-TOF, polymers, Tween 20, Polysorbate 20, complex mixtures, isobaric compounds, resolving power

Toshiji Kudo[1], Volker Sauerland[2], Arndt Asperger[2]; [1]Bruker Japan K.K., Daltonics Division, Yokohama-shi, Kanagawa, Japan; [2]Bruker Daltonics GmbH & Co. KG, Bremen, Germany.

Introduction

Polysorbates are nonionic surfactants widely used in pharmaceutical, cosmetic, and food applications. Due to their amphiphilic nature and stabilizing properties, detailed characterization of polysorbates is increasingly important for product quality, stability assessment, and regulatory compliance. However, polysorbates are inherently heterogeneous materials comprising complex oligomer mixtures and remain, therefore, a challenging class of analytes.

Polysorbate 20 (also known as Tween 20) is synthesized by etherification of sorbitan with polyethylene glycol (PEG) followed by esterification of the terminal hydroxyl groups with fatty acids. The resulting Polysorbate 20 product contains a mixture of oligomers differing in PEG and acyl chain length, respectively, as well as presence or absence of the sorbitan core. This level of sample complexity results in highly congested mass spectra and, thus, requires a high level of analytical performance, resolving power in particular, when aiming for comprehensive structural characterization of Polysorbate 20 by direct mass spectrometry (MS) analysis.

This application note highlights the merits of the neofleX benchtop MALDI-TOF mass spectrometer for the detailed characterization of complex polymer mixtures. Polysorbate 20 served as an ideal test case to demonstrate the benefits of neofleX resolving power and mass accuracy as the main performance indicators when aiming for separation of closely spaced isobaric oligomer species. Data shown here illustrate the unique capabilities neofleX provides for simple and fast, though comprehensive characterization of complex polymer samples making it an ideal tool for rapid-turnaround synthesis verification, stability assessment and quality control.

Experimental

Sample preparation

Polysorbate 20 (purchased from Sigma-Aldrich) was dissolved in THF at a concentration of 20 mg/mL.

trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile, DCTB (purchased from either Sigma-Aldrich or Tokyo Chemical Industry Co. Ltd.) was used as the MALDI matrix. The matrix solution was prepared at a concentration of 20 mg/mL in THF.

NaTFA (2 mg/mL in THF) was used as a cationizing salt.

Polysorbate 20, DCTB matrix and NaTFA solutions were mixed in a volume ratio 1:20:1. An aliquot of the resulting mixture was applied onto an MTP 384 ground steel MALDI target plate, and was allowed to dry under ambient conditions.

MALDI-TOF analysis

Mass spectra were acquired on a Bruker neofleX benchtop MALDI-TOF mass spectrometer. Measurements were performed in positive reflector mode covering a detection range 600-2500 *m/z*. Adduct ions $[M+Na]^+$ were observed as the dominating ion species. External *m/z* calibration was performed for method precalibration using fleXstandard Polymers (Bruker) as a reference substance.

Data processing and analysis

MS data were internally recalibrated in Bruker flexAnalysis 5.1 using Polysorbate 20 oligomer signals of known identity as internal calibrants.

Data analysis was performed in Polymerix 3.0 software (Sierra Analytics, Inc.) using the Homopolymer Analysis mode and applying a matching tolerance of 0.01 *m/z*. Polymerix, when operated in Homopolymer Analysis mode, expects linear polymer structures consisting of a single repeat unit and two terminal groups. However, Polysorbate 20 represents a complex mixture of non-linear, multi-branched oligomer series as shown in Figure 1.

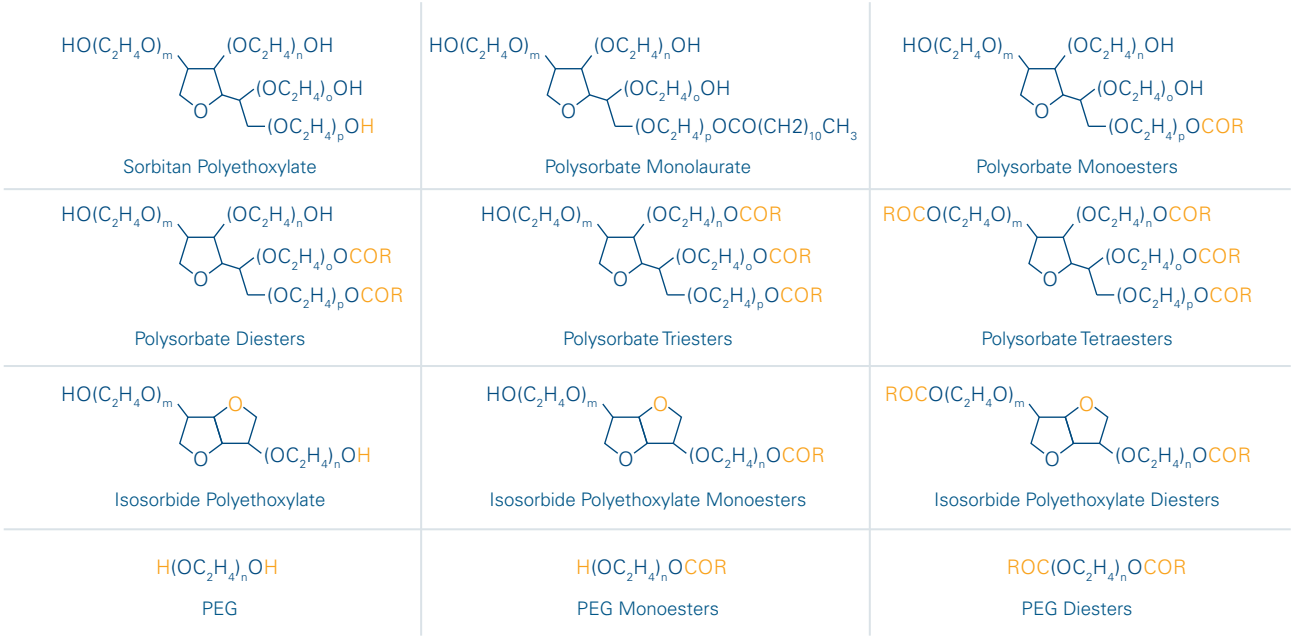


Figure 1. **Putative molecular structures of oligomer series contributing to the heterogeneity of Polysorbate 20.**
Polysorbate monolaurate is expected to represent the main oligomer series accompanied by a variety of structurally related species. R denotes acyl chain moieties. m, n, o and p denote the number of ethylene glycol repeat units in the PEG polymer moiety.

Homopolymer Series Definition

+ +	Enabled	Label	Alpha End Group	Repeat	Omega End Group	Charge State	Adduct	Adduct Charge	Loss	Adjust For Adduct/Loss	Low Mass	High Mass
--	☑	S1	Sorbitan	Ethylene Glycol		1	Na	1		☑	1.0	100000.0
--	☑	S2	Sorbitan	Ethylene Glycol	Oc	1	Na	1	Water	☑	1.0	100000.0
--	☑	S3	Sorbitan	Ethylene Glycol	Dec	1	Na	1	Water	☑	1.0	100000.0
--	☑	S4	Sorbitan	Ethylene Glycol	Lau	1	Na	1	Water	☑	1.0	100000.0
--	☑	S5	Sorbitan	Ethylene Glycol	Myr	1	Na	1	Water	☑	1.0	100000.0
--	☑	S6	Sorbitan	Ethylene Glycol	Pam	1	Na	1	Water	☑	1.0	100000.0
--	☑	S7	Sorbitan	Ethylene Glycol	Ole	1	Na	1	Water	☑	1.0	100000.0
--	☑	S8	Sorbitan	Ethylene Glycol	LauLau	1	Na	1	2 Water	☑	1.0	100000.0
--	☑	S9	Sorbitan	Ethylene Glycol	LauMyr	1	Na	1	2 Water	☑	1.0	100000.0
--	☑	S10	Sorbitan	Ethylene Glycol	LauPam	1	Na	1	2 Water	☑	1.0	100000.0
--	☑	S11	Sorbitan	Ethylene Glycol	LauSte	1	Na	1	2 Water	☑	1.0	100000.0
--	☑	S12	Sorbitan	Ethylene Glycol	MyrPam	1	Na	1	2 Water	☑	1.0	100000.0
--	☑	S13	Sorbitan	Ethylene Glycol	MyrSte	1	Na	1	2 Water	☑	1.0	100000.0
--	☑	S14	Sorbitan	Ethylene Glycol	PamSte	1	Na	1	2 Water	☑	1.0	100000.0
--	☑	S15	Sorbitan	Ethylene Glycol	LauLauLau	1	Na	1	3 Water	☑	1.0	100000.0
--	☑	S16	Sorbitan	Ethylene Glycol	LauLauMyr	1	Na	1	3 Water	☑	1.0	100000.0
--	☑	S17	Sorbitan	Ethylene Glycol	LauLauPam	1	Na	1	3 Water	☑	1.0	100000.0
--	☑	S18	Sorbitan	Ethylene Glycol	LauLauSte	1	Na	1	3 Water	☑	1.0	100000.0
--	☑	S19	Isosorbide	Ethylene Glycol		1	Na	1		☑	1.0	100000.0
--	☑	S20	Isosorbide	Ethylene Glycol	Lau	1	Na	1	Water	☑	1.0	100000.0
--	☑	S21	Isosorbide	Ethylene Glycol	Myr	1	Na	1	Water	☑	1.0	100000.0
--	☑	S22	Isosorbide	Ethylene Glycol	Ole	1	Na	1	Water	☑	1.0	100000.0
--	☑	S23	Isosorbide	Ethylene Glycol	Ste	1	Na	1	Water	☑	1.0	100000.0
--	☑	S24	Isosorbide	Ethylene Glycol	LauLau	1	Na	1	2 Water	☑	1.0	100000.0
--	☑	S25		Ethylene Glycol	Water	1	Na	1		☑	1.0	100000.0
--	☑	S26		Ethylene Glycol	Lau	1	Na	1		☑	1.0	100000.0
--	☑	S27		Ethylene Glycol	Myr	1	Na	1		☑	1.0	100000.0
--	☑	S28		Ethylene Glycol	Ole	1	Na	1		☑	1.0	100000.0
--	☑	S29		Ethylene Glycol	LauMyr	1	Na	1	Water	☑	1.0	100000.0
--	☑	S30		Ethylene Glycol	LauSte	1	Na	1	Water	☑	1.0	100000.0

Figure 2. **Homopolymer series definitions set up in Polymerix software for the analysis of Polysorbate 20 MALDI-TOF data.**
Abbreviations: Lau - lauric acid, Oc - caprylic acid, Dec - capric acid, Myr - myristic acid, Pam - palmitic acid, Ole - oleic acid, Ste - stearic acid.

To set up Polymerix for correct annotation of MALDI-TOF MS spectra acquired from Polysorbate 20, the following homopolymer series definitions were specified in the software:

- **Repeat unit:** Ethylene glycol (EG)
- **Alpha-end group:** Sorbitan-derived structure
- **Omega-end group:** Total fatty acid moiety
- **Loss:** Water (number of water losses depending on number of acyl chains in the molecule)

A total of 30 oligomer series were defined to cover the compositional heterogeneity of Polysorbate 20. Figure 2 provides the list of series definitions used in this study considering two different sorbitan core structures as well as differing numbers and chain lengths of incorporated fatty acids ranging from caprylic (C_8) up to oleic/stearic acid (C_{18}). Isomeric fatty acid moieties, such as [lauric acid + palmitic acid] versus [myristic acid + myristic acid], being identical in their mass, were considered as a single list entry (e.g. LauPam).

Results

Deconvoluting the complexity of Polysorbate 20 with neofleX MALDI-TOF

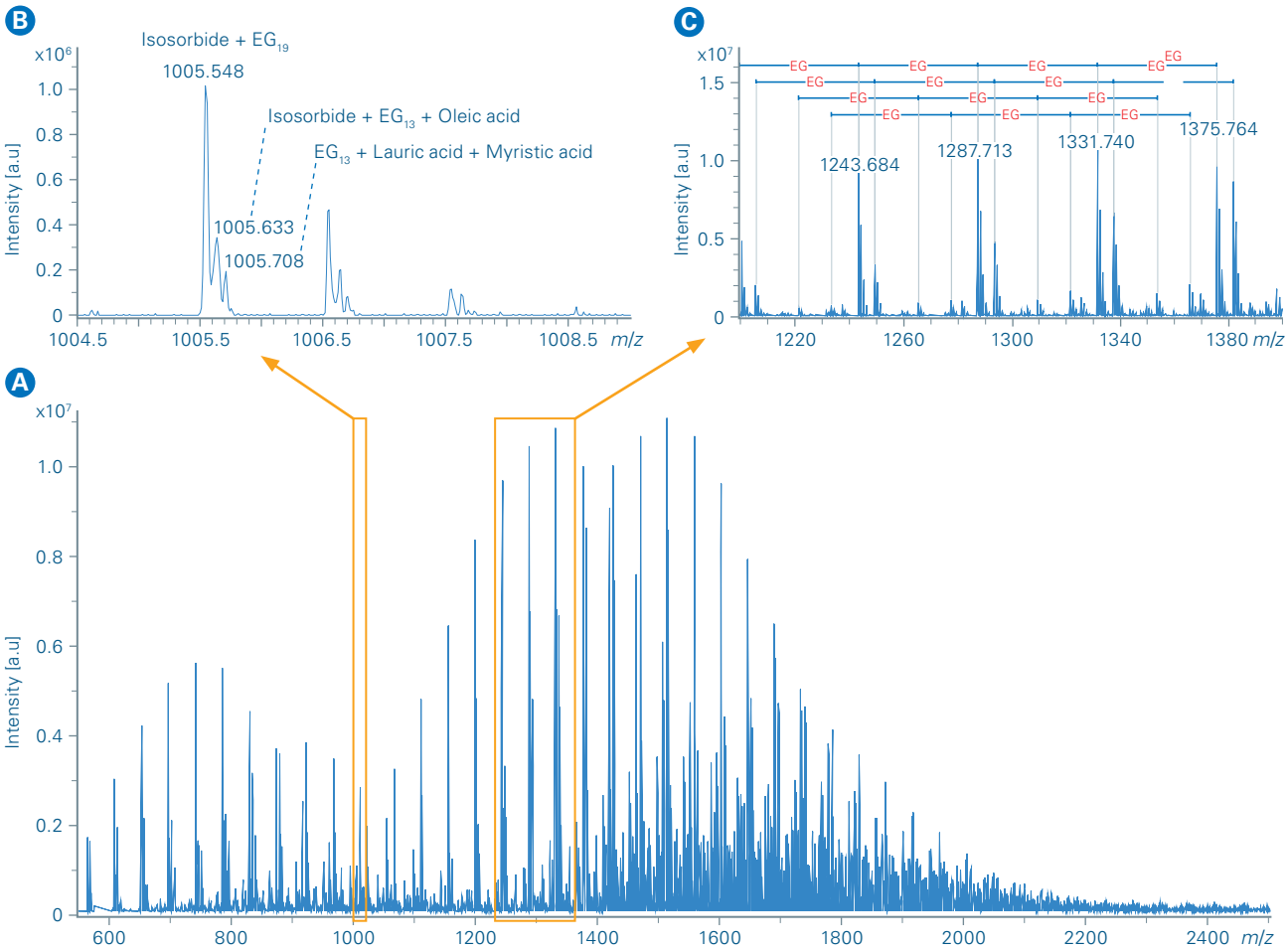


Figure 3. MALDI-TOF spectrum of Polysorbate 20.
Ⓐ Total spectrum view. The spectrum features an extremely high signal density reflecting the intrinsic heterogeneity of the sample. Expanded view on *m/z* region 1004-1008. Ⓒ Expanded view on *m/z* region 1220-1400. Annotations in red color indicate 44 *m/z* distances corresponding to the ethylene glycol (EG) repeat unit (C_2H_4O). Detection of multiple series featuring EG specific distances, but shifted in mass, indicates the presence of a range of oligomer series differing in the type and number of fatty acid molecules incorporated as well as in the presence/absence of sorbitan and isosorbide core, respectively. Ⓑ Isobaric oligomer species featuring mass differences as little as 80 mDa are separated. These isobaric components appear clearly resolved as distinct peaks providing information at a level of detail that cannot be achieved in low-resolution, linear-mode MALDI-TOF measurements. The outstanding resolving power provided by neofleX (up to R=30,000) is, therefore, essential for the analysis of complex polymer mixtures, in which minor mass differences can indicate fundamental differences with regard to chemical structure.

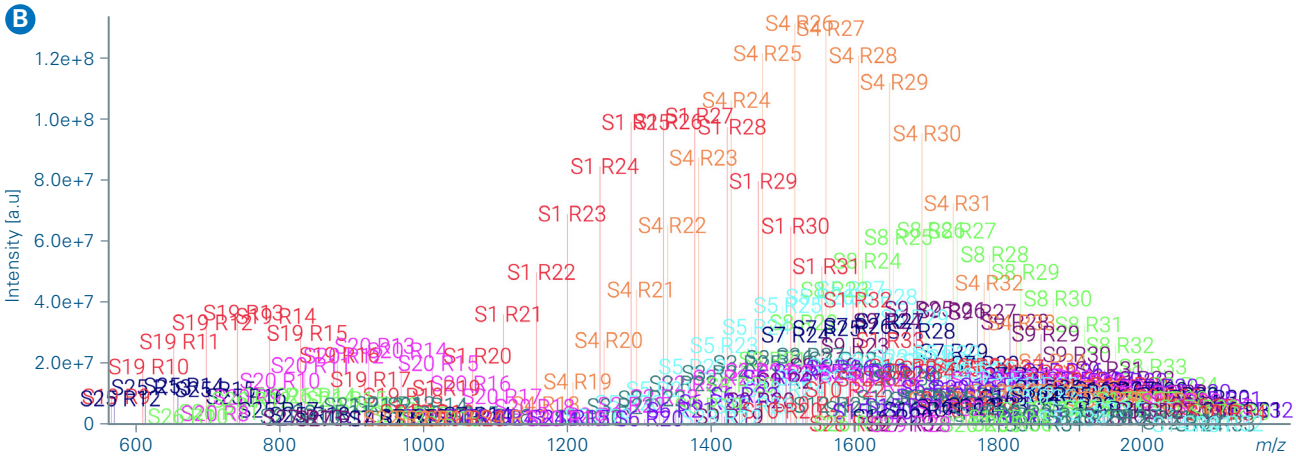
Automated assignment of Polysorbate 20 using Polymerix software

Ⓐ

Homopolymer Results Summary

Series Label	Mn	Mw	Mz	PD	DPn	DPw	DPz	Percent Series	Percent Spectrum	Alpha End Group	Repeat	Omega End Group	Charge State	Adduct	Loss
Total/Average	1516.691	1530.815	1544.721	1.010	25.403	25.724	26.040	100.00	97.73		C2H4O		1	Na	
S1	1338.068	1356.855	1375.351	1.014	26.666	27.093	27.513	17.04	16.65	C6H12O5	C2H4O		1	Na	
S2	1477.029	1485.917	1494.662	1.006	26.549	26.751	26.949	2.31	2.25	C6H12O5	C2H4O	C8H16O2	1	Na	H2O
S3	1506.781	1517.840	1528.713	1.007	26.588	26.839	27.086	3.19	3.11	C6H12O5	C2H4O	C10H20O2	1	Na	H2O
S4	1540.440	1556.864	1573.037	1.011	26.716	27.089	27.456	22.07	21.56	C6H12O5	C2H4O	C12H24O2	1	Na	H2O
S5	1559.896	1572.212	1584.326	1.008	26.521	26.801	27.076	6.37	6.23	C6H12O5	C2H4O	C14H28O2	1	Na	H2O
S6	1575.574	1584.900	1594.069	1.006	26.240	26.452	26.660	2.44	2.39	C6H12O5	C2H4O	C16H32O2	1	Na	H2O
S7	1637.611	1655.273	1669.536	1.011	27.059	27.460	27.784	4.39	4.29	C6H12O5	C2H4O	C18H34O2	1	Na	H2O
S8	1737.327	1750.670	1763.902	1.008	26.641	26.944	27.245	10.47	10.23	C6H12O5	C2H4O	(C12H24O2)2	1	Na	H4O2
S9	1761.232	1773.991	1786.688	1.007	26.547	26.837	27.125	6.15	6.01	C6H12O5	C2H4O	(C12H24O2)(C14H28O2)	1	Na	H4O2
S10	1795.488	1808.313	1821.108	1.007	26.689	26.980	27.271	3.28	3.21	C6H12O5	C2H4O	(C12H24O2)(C16H32O2)	1	Na	H4O2
S11	1916.938	1921.430	1925.974	1.002	28.856	28.958	29.062	1.65	1.61	C6H12O5	C2H4O	(C12H24O2)(C18H34O2)	1	Na	H4O2
S12	1744.198	1753.318	1762.808	1.005	24.887	25.094	25.310	0.95	0.93	C6H12O5	C2H4O	(C14H28O2)(C16H32O2)	1	Na	H4O2
S13	1890.770	1896.551	1902.346	1.003	27.625	27.757	27.888	1.36	1.33	C6H12O5	C2H4O	(C14H28O2)(C18H34O2)	1	Na	H4O2
S14	2100.409	2100.601	2100.791	1.000	31.704	31.709	31.713	0.01	0.01	C6H12O5	C2H4O	(C16H32O2)(C18H36O2)	1	Na	H4O2
S15	1917.275	1926.903	1936.397	1.005	26.182	26.400	26.616	2.46	2.41	C6H12O5	C2H4O	(C12H24O2)3	1	Na	H6O3
S16	1944.328	1952.693	1960.849	1.004	26.159	26.349	26.535	1.64	1.60	C6H12O5	C2H4O	(C12H24O2)2(C14H28O2)	1	Na	H6O3
S17	2002.243	2006.034	2009.777	1.002	26.838	26.924	27.009	0.74	0.72	C6H12O5	C2H4O	(C12H24O2)2(C16H32O2)	1	Na	H6O3
S18	2044.817	2045.356	2045.893	1.000	27.168	27.181	27.193	0.19	0.19	C6H12O5	C2H4O	(C12H24O2)2(C18H36O2)	1	Na	H6O3
S19	740.730	756.306	771.775	1.021	13.507	13.861	14.212	4.26	4.16	C6H10O4	C2H4O		1	Na	
S20	925.046	937.938	950.617	1.014	13.147	13.440	13.728	2.95	2.88	C6H10O4	C2H4O	C12H24O2	1	Na	H2O
S21	944.299	950.020	955.687	1.006	12.948	13.078	13.206	0.54	0.53	C6H10O4	C2H4O	C14H28O2	1	Na	H2O
S22	978.894	979.389	979.883	1.001	12.506	12.517	12.528	0.06	0.06	C6H10O4	C2H4O	C18H34O2	1	Na	H2O
S23	1680.955	1691.837	1702.707	1.006	28.406	28.654	28.900	3.20	3.13	C6H10O4	C2H4O	C18H36O2	1	Na	H2O
S24	1115.816	1119.174	1122.523	1.003	12.933	13.010	13.086	0.20	0.19	C6H10O4	C2H4O	(C12H24O2)2	1	Na	H4O2
S25	653.508	661.863	670.313	1.013	14.435	14.624	14.816	1.08	1.06		C2H4O	H2O	1	Na	
S26	815.767	833.212	858.530	1.021	13.982	14.379	14.954	0.93	0.90		C2H4O	C12H24O2	1	Na	
S27	861.972	898.333	961.002	1.042	14.395	15.221	16.644	0.07	0.07		C2H4O	C14H28O2	1	Na	
S28	1559.008	1559.008	1559.008	1.000	29.000	29.000	29.000	0.00	0.00		C2H4O	C18H34O2	1	Na	
S29	1661.115	1661.115	1661.115	1.000	28.000	28.000	28.000	0.00	0.00		C2H4O	(C12H24O2)(C14H28O2)	1	Na	H2O
S30	1847.236	1847.236	1847.236	1.000	31.000	31.000	31.000	0.00	0.00		C2H4O	(C12H24O2)(C18H34O2)	1	Na	H2O

Ⓑ



Ⓒ

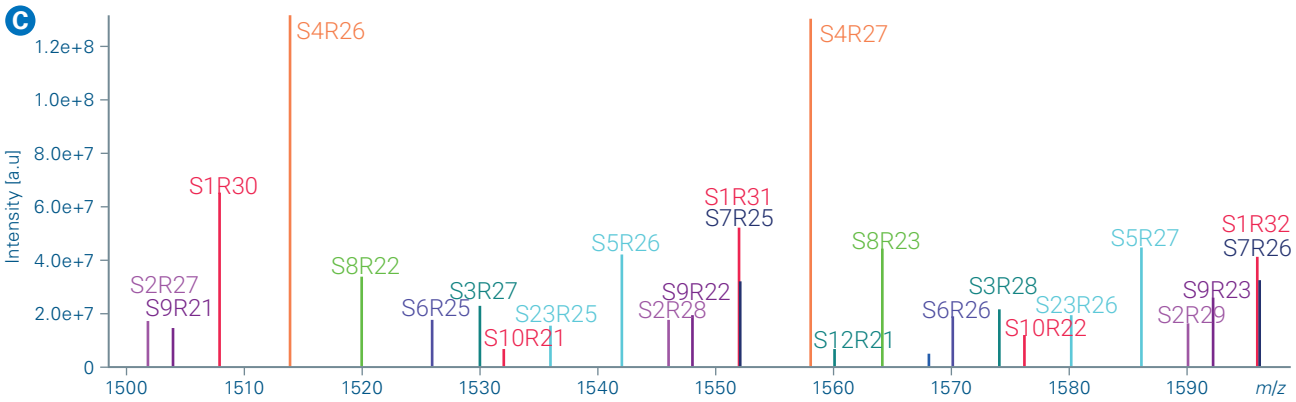


Figure 4. MALDI-TOF spectrum of Polysorbate 20 analyzed in Polymerix software.
Ⓐ Analysis result table. Ⓑ Total view on MALDI-TOF spectrum annotated with matching Polysorbate 20 oligomer series based on the definitions listed in Figure 2. R denotes the number of ethylene glycol repeat units. Ⓒ Zoomed view on MALDI-TOF spectrum region 1500–1600 *m/z* annotated with matching Polysorbate 20 oligomer species based on the series definitions listed in Figure 2. 97.73% of the signals detected in the MALDI-TOF spectrum were successfully assigned. This outstanding spectral coverage underlines the excellent data quality delivered by neofleX allowing for simple and fast, though comprehensive characterization of complex polymer mixtures.

Conclusion

- neofleX benchtop MALDI-TOF is an ideally suited platform for simple and fast, though comprehensive characterization of complex polymer mixtures such as Polysorbate 20.
- neofleX resolving power (up to R=30,000) allows for deconvolution of structurally different oligomer series, including isobaric species, in a single MALDI-TOF spectrum providing detailed insight in the highly complex composition of Polysorbate 20.
- Polymerix software streamlines the interpretation of highly congested MALDI-TOF mass spectra of complex polymer samples through automated assignment and visualization of oligomer series.
- neofleX benchtop MALDI-TOF in combination with Polymerix software provides an efficient platform for rapid-turnaround quality control, formulation studies, synthesis verification, and stability assessment accelerating macromolecular research as well as the development of new functional polymer materials.

Bruker Daltonics is continually improving its products and reserves the right to change specifications without notice. © Bruker Daltonics 08-2026, MT-150, 1930697

For Research Use Only. Not for use in clinical diagnostic procedures.

Bruker Switzerland AG

Fällanden · Switzerland
Phone +41 44 825 91 11

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

www.linkedin.com/company/bruker-daltonics
info.ms@bruker.com – www.bruker.com

Learn more at **www.bruker.com/neoflex**