SHIMADZU
Excellence in Science

Application News

No. B77

MALDI-TOF Mass Spectrometry

Characterisation of a PEG Conjugated Medicine; Polymer Analysis Using the MALDI-8020 Benchtop Linear MALDI-TOF Mass Spectrometer

Polymers are molecules made of multiply repeated subunits called monomers, where physical and chemical properties of each play important roles and are employed in different fields such as medical, pharmaceutical, industrial and material sciences. Among several analytical techniques that can be used for polymer analysis, including SEC/GPC chromatography and NMR/FT-IR spectroscopy, MALDI mass spectrometry is very popular due to its ability to quickly provide information on molecular mass distribution and polydispersity, as well as determination of start- and end- groups to help guide manufacturing and QC laboratories.

Polymers are anticipated to be applied in therapeutics as carrier molecules for controlled and targeted delivery of pharmaceutical agents. D-α-tocopherol (vitamin E) tethered to polyethylene glycol (PEG) 1000 succinate (vitamin E-TPGS, Fig. 1) has been proven to enhance the drug solubility, permeability and stability.

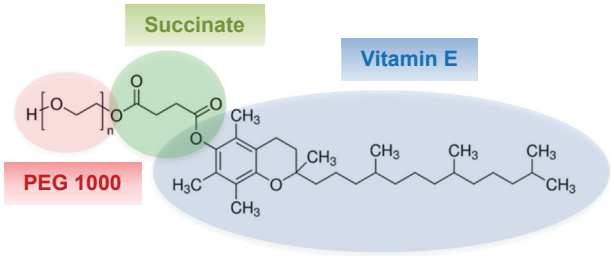

Fig. 1 Structure of Vitamin E-TPGS

Here, we provide a complete solution for the characterisation of vitamin E-TPGS polymer sample (Fig. 2), which combines the use of MALDI-8020 benchtop linear MALDI-TOF mass spectrometer and Polymerix polymer data analysis software (Sierra Analytics).

Simona Salivo, Tom K. Abban, Matthew E. Openshaw, Ei-ichi Matsuo

Samples and Method

Vitamin E-TPGS was purchased from Sigma-Aldrich. The sample solution (2 mg/mL, 1:1 acetonitrile/water) was mixed with the MALDI matrix solution (alpha-cyano-4-hydroxycinnamic acid (CHCA), 10 mg/mL, 1:1 acetonitrile/water). Sodium chloride solution (1 mM, 1:1 acetonitrile/water) was used as a cationisation agent to pre-coat the MALDI target. The mixed solution of sample and matrix (1 µL) was spotted on top of the salt layer.

The acquisition parameters of the MALDI-MS analyses are summarised in Table 1.

Table 1 MALDI-MS Data Acquisition Parameters

Tuning	linear
Polarity	positive
Mass range	100-3000 Da
Laser rep. rate	200 Hz
Accumulation rate (shots/profile)	50
Profiles	200

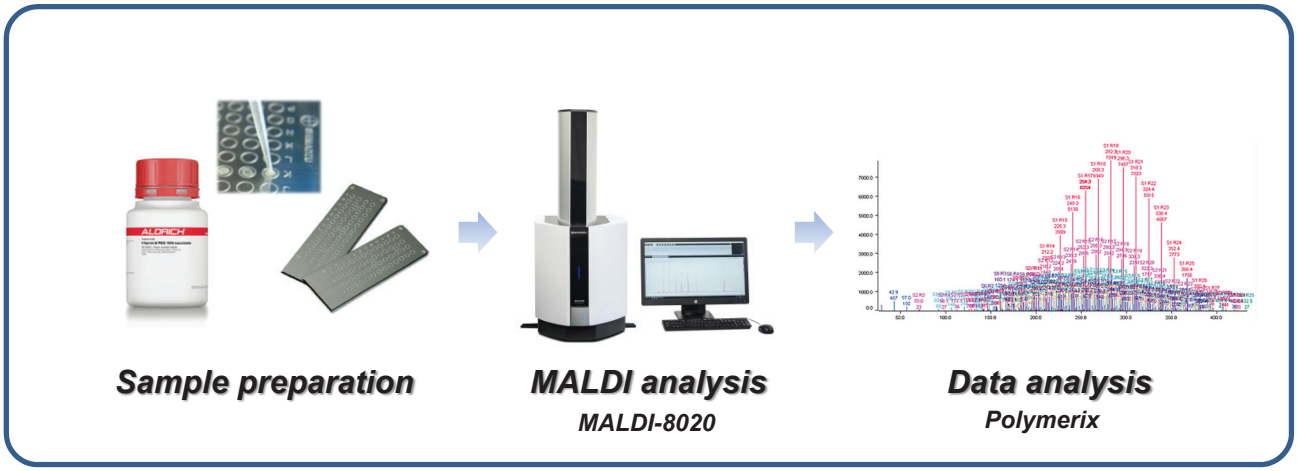

Fig. 2 Workflow for the Characterisation of Vitamin E-TPGS Polymer.

Results

Fig. 3 shows the MALDI-MS spectrum of sodiated vitamin E-TPGS. The main polymer ion series is visible in the range of *m/z* 1100-1900. Two minor ion series are also observed (*m/z* 800-1200 and 1700-2200, Fig. 3 top panel).

Fig. 3 (bottom panel) shows two peaks spaced by 44 Da, consistent with the expected mass of the repeating unit (C_2H_4O), corresponding to two polymer chains differing in length by one PEG monomer. The monoisotopic peaks of the two PEG units show good resolution values (r values in { }).

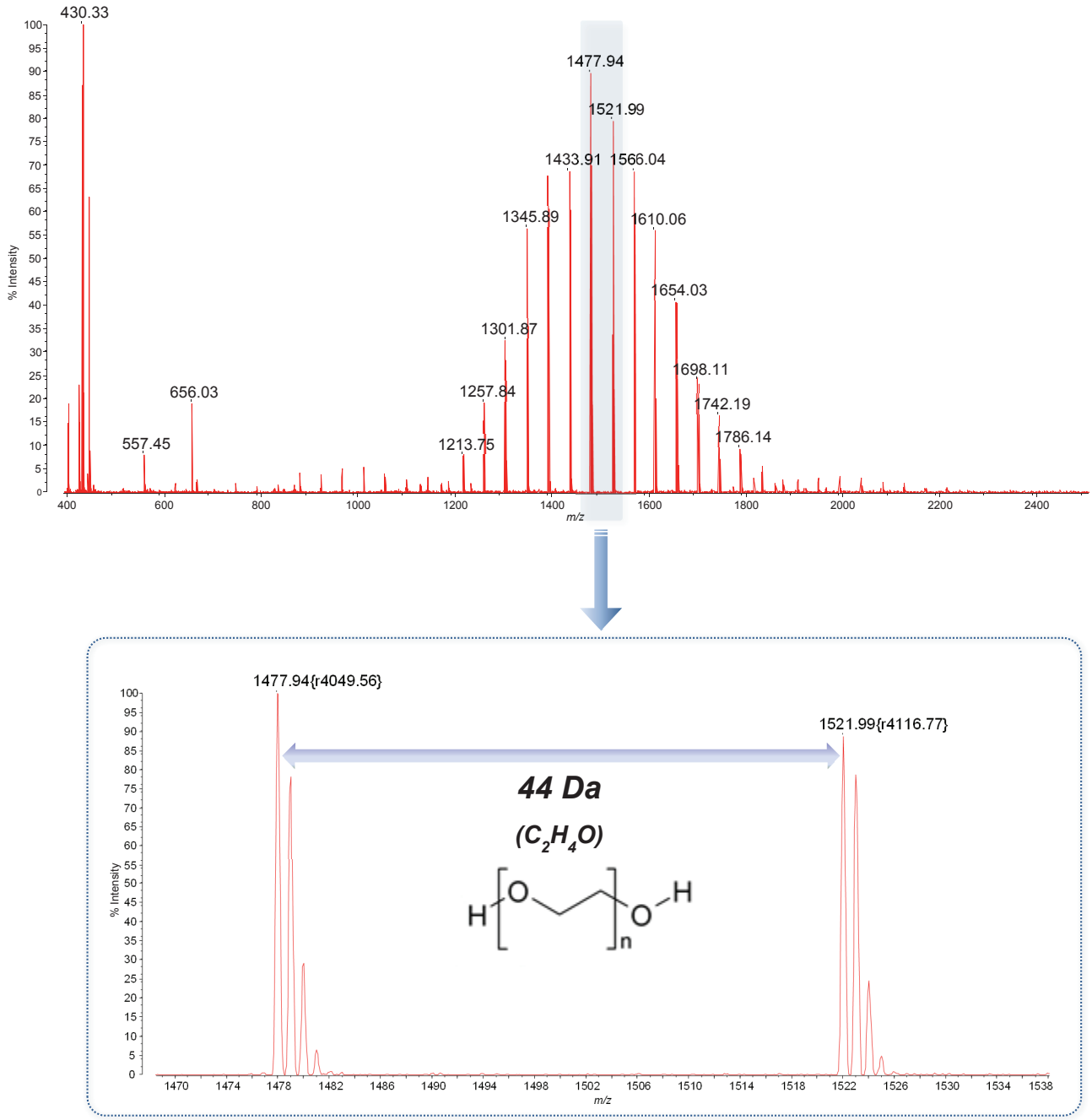

Fig. 3 MALDI-MS Spectrum of Sodiated Vitamin E-TPGS Polymer (Top Panel). The Bottom Panel is the Magnified View of the Blue Hatching Part.

Two ions at *m/z* 430.33 and 557.45, detected in the mass spectrum of sodiated vitamin E-TPGS (Fig. 3), are consistent with the proposed structures of vitamin E (radical ion) and vitamin E succinate plus the $(CH_2)_2$ moiety of the first PEG unit, respectively (Fig. 4 *a* and *b*).

Fig. 5 shows the structures and formulae of the proposed end groups which were used for subsequent homopolymer series definitions (Fig. 6) using the Polymerix software.

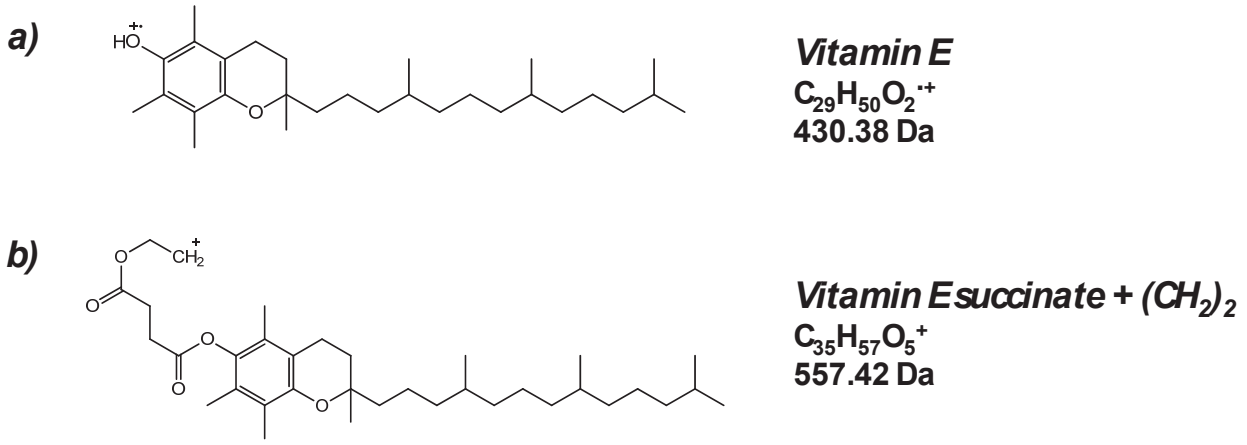

Fig. 4 a) Chemical Structure and Formula of Vitamin E (radical ion); b) Chemical Structure and Formula of Vitamin E succinate Plus Partial PEG Unit.

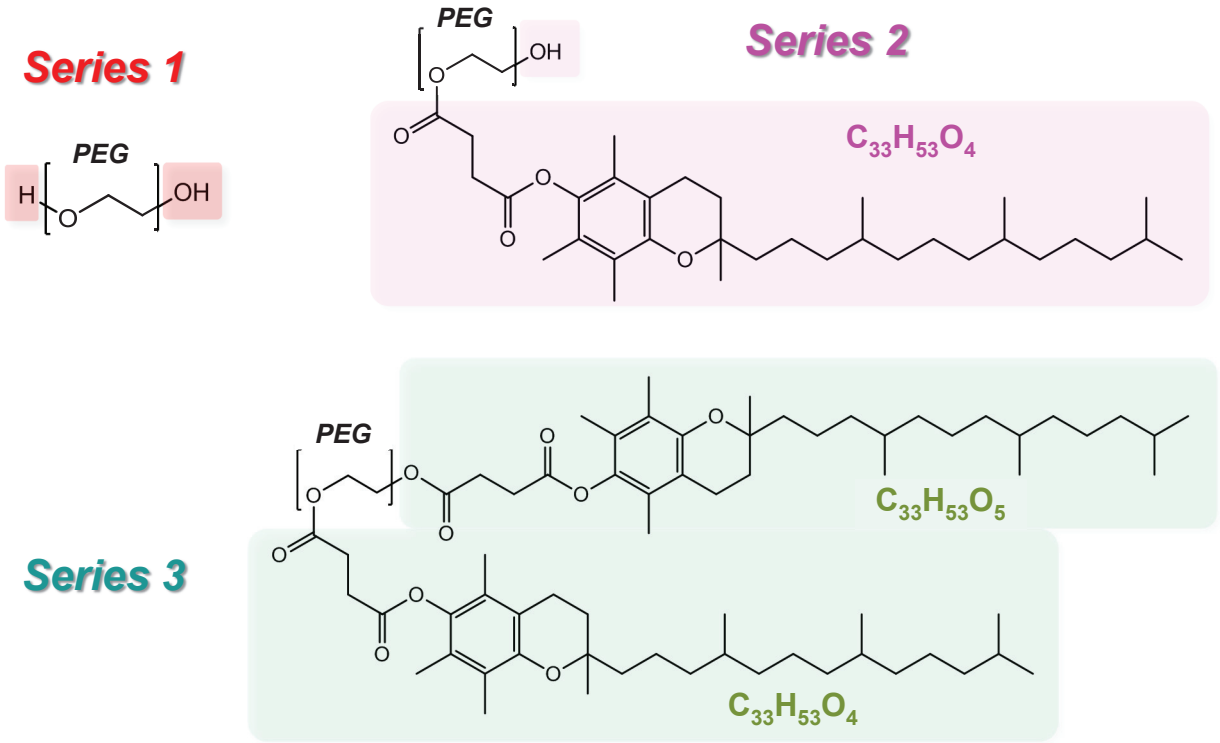

Fig. 5 Chemical Structures and Formulae of Proposed End Groups for the Homopolymer Series Calculation.

Three series (Series 1 - 3) were computed using the proposed end groups (Fig. 6), showing a low residual mass error (excess mass) and thus a good fit between each hypothetical polymer formula and input data (reference mass).

The homopolymer assignments plot is shown in Fig. 7. The target mass spectrum is presented with all matched series peaks labeled by each series color.

	Enabled	Excess Mass	Monoisotopic m/z 1	Monoisotopic m/z 2	Alpha End Group	Repeat	Omega End Group	Charge State	Adduct	Adduct Charge	Loss	Low Mass	High Mass
Series 1	☑	0.02970	965.5800	1009.6200	OH	C2H4O	H	1	Na	1		1.0	100000.0
Series 2	☑	0.00314	1477.9400	1521.9900	OH	C2H4O	C33H53O4	1	Na	1		1.0	100000.0
Series 3	☑	0.04658	1990.3700	2034.3700	C33H53O5	C2H4O	C33H53O4	1	Na	1		1.0	100000.0

Fig. 6 Homopolymer Series Definition Table (Polymerix Software)

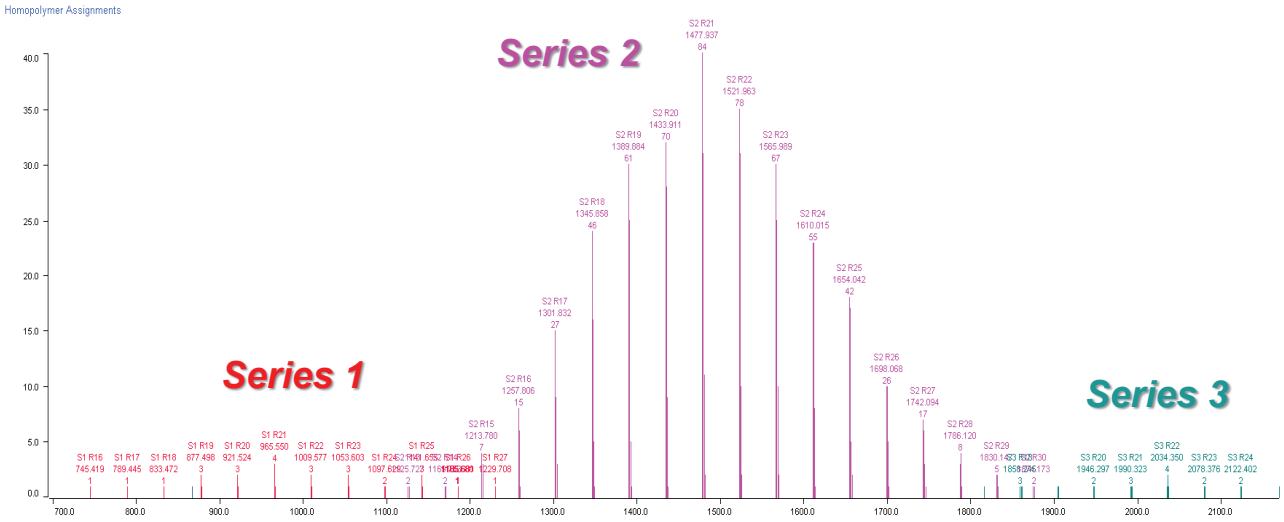

Fig. 7 Homopolymer Assignments Plot Generated from the Series Definition Using Polymerix Software.

First Edition: Mar. 2018

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/