EVONIK
Leading Beyond Chemistry

Focus Analytics

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ANALYTICS OF BIOMOLECULES AND POLYMERS –

MALDI-TOF mass spectrometry

With laboratories in Hanau, Marl, Darmstadt, and Shanghai, the analytics team of Evonik provides high-quality quantitative and qualitative analyses using organic mass spectrometry (MS). Our experts have both low- and high-resolution mass spectrometers combined with gas or liquid chromatography at their disposal.

At the end of 2021, the MS laboratory in Hanau acquired a MALDI-TOF mass spectrometer for the analysis of polymers and biomolecules.

The ionisation technique combined with mass determination in the time-of-flight mass spectrometer opens up new possibilities for investigating large molecules such as intact proteins and/or polymer distributions up to approx. 100 kDa.

Figure 1: Metal carrier (target) ready for matrix preparation

THE PRINCIPLE

MALDI stands for "matrix-assisted laser desorption ionisation" and TOF for "time of flight" (i.e. the flight time of the ions released). For this analytical technique, the sample is first crystallised together with an auxiliary matrix on a carrier (target) (Figure 1). The auxiliary matrix is able to absorb UV light and transfer its energy to the sample. It also transfers electrical charges (e.g. through protonation). For analysis, the carrier plate with the prepared sample is fed into the device and irradiated with a UV laser. The matrix is vaporised, and the energy of the laser is transferred to the sample. The sample is then ionised and transported into the gas phase (Figure 2).

The molecular ions are then accelerated into an electric field and strike the detector after a flight distance of approx. 1.5 m.

By measuring the time of flight between the laser pulse and the detector deflection, the molecular weight can be determined: larger molecular ions fly more slowly and thus take longer to reach the detector than smaller molecular ions (see Figure 3).

THE ADVANTAGES OF THE AUXILIARY MATRIX

A major advantage of MALDI-TOF-MS is the gentle ionisation and release of the molecular ions because of the indirect energy transfer through the auxiliary matrix. The matrix can be selected according to the chemical properties of the sample. Molecules that cannot be ionised in any other way can thus be analysed.

The gentle ionisation usually produces only a small number of charges. Even large molecules are usually still detected as singly charged molecular ions. This greatly simplifies the evaluation of the mass spectra and is particularly advantageous for molecules that involve a synthesis- and/or raw material-dependent polymer distribution. With electrospray ionisation (ESI), such spectra can practically no longer be evaluated because of the additional superposition of different charge states.

With MALDI, the mass spectra remain clear, and a polymer distribution can be visualised as such (Figure 4).

MALDI-TOF PRINCIPLE

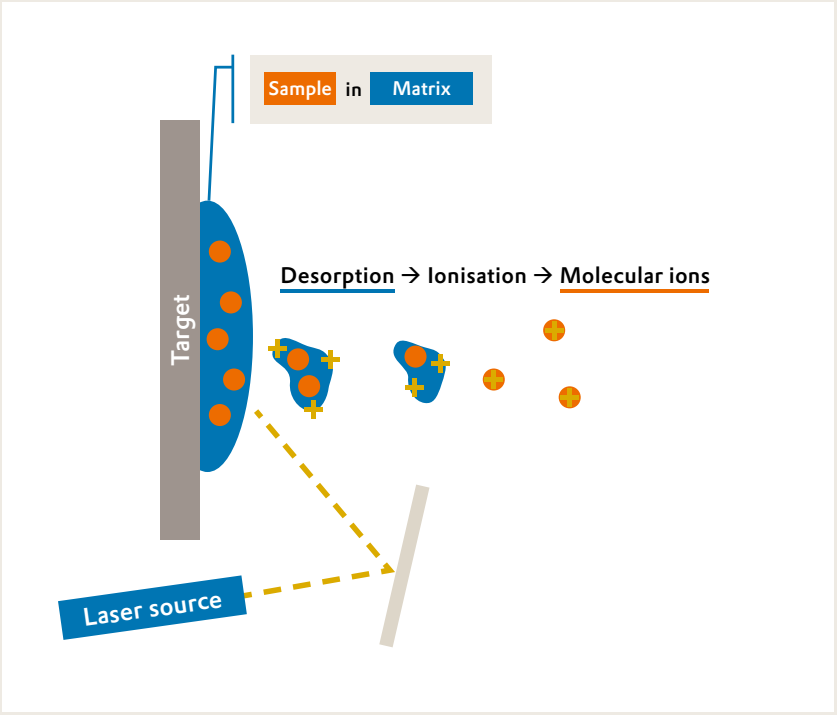

Figure 2: Principle of the ionisation of molecules in the sample matrix in MALDI-TOF

THE APPROPRIATE MEASURING MODE

Depending on what needs to be analysed,

- measurements can be made in linear mode ("unlimited" measurement time = detection of all ions – even with high molecular weight) with lower resolution or
- in reflector mode (limited measurement time = not all ions are detected) with higher resolution because of the longer flight distance.

For example, a mass precision of up to 10 ppm can be achieved with smaller molecular weights of up to approx. 5 kDa and sufficient protonable positions. The choice of mode thus also depends on the samples and their target molecular weight.

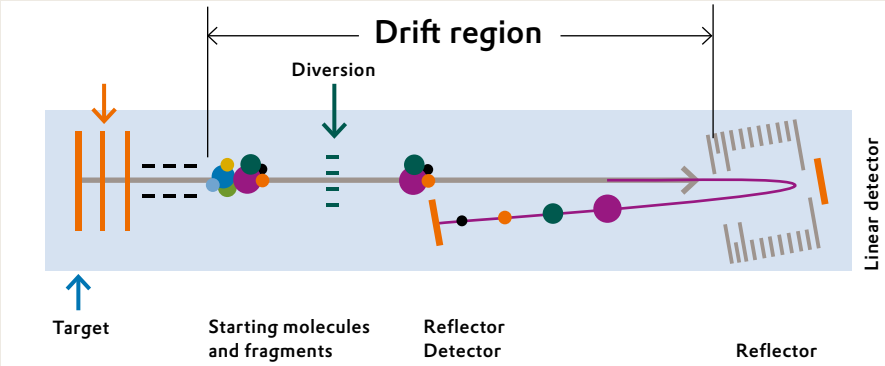

Figure 3: Schematic representation of the ion separation in MALDI-TOF-MS because of the different lengths of the time of flight.

GENERAL USE OF THE ANALYSIS TECHNIQUE

Peptides/Proteins

The technique is associated primarily with the analysis of peptides and proteins. Applications such as the identification of pathogens in hospitals now predominate in routine operation.

This is where the extremely short analysis times required to screen the samples come into play. Only minute sample quantities in the fmol range are required (e.g. from protein digests of spots from 2D gels). One well-known example is the automated "High throughput peptide mapping" in proteome analysis applications. This enables a rapid database analysis and assignment via the single charge in the "fingerprint region".

Polymers

The gentle ionisation makes this technique particularly interesting for analysing polymers (e.g. polyethylene glycols, organomodified polydimethylsiloxanes). The polymer chains are not fragmented and can thus be analysed as a whole. Molecular weights up to over 100 kDa can thus be measured.

Depending on the average molecular weight distribution, the polymers themselves (or modifications thereof) can still be identified even in mixtures such as polyethylene glycol (Fig. 4) up to a molecular weight of approx. 20 kDa.

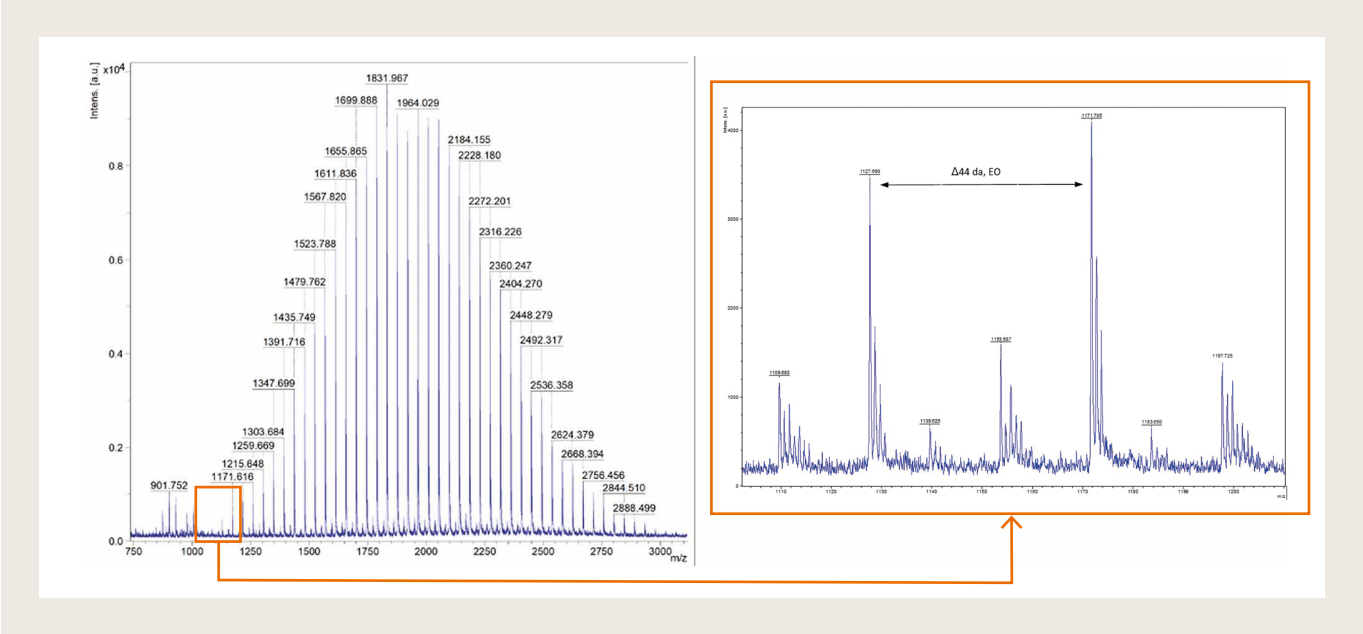

Figure 4: Example of a high-resolution MALDI-TOF mass spectrum of a polyethylene glycol. Overview spectrum (left) and zoom (right).

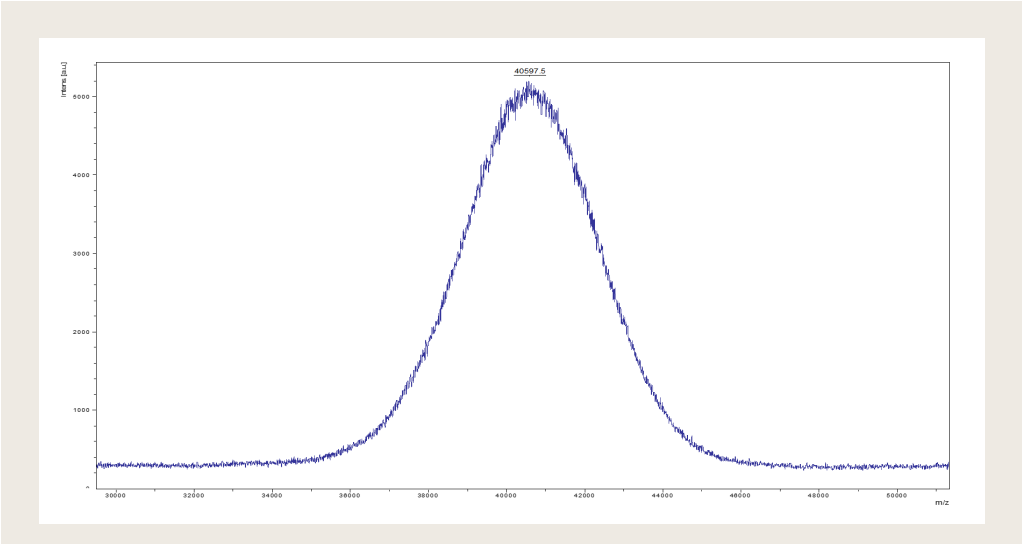

Figure 5: Representation of the MS signal of a polymer at low resolution

With molecular weights greater than approx. 20 kDa, the characteristic curve from gel permeation chromatography (GPC) is obtained with low resolution. With increasingly superimposed signals of the polymer distribution, only the average molar mass (see Figure 5) can be determined.

Because polymers are now playing an increasingly important role, especially in pharmaceuticals, the device was qualified under good manufacturing practice (GMP) specifically for this quality status. Thus, after a corresponding product-specific method development and validation, the analysis can be offered for the pharmaceutical sector also under GMP regulatory requirements. MALDI-TOF-MS complements our range of mass spectrometry and allows us to analyse complex large molecules. Among other things, the technique is characterised by its variability in sample preparation, more options to analyse poorly ionisable molecules, and the short measurement times.

The mass spectrometry team in Hanau, led by Dr Jürgen Volz, looks forward to helping you answer your research questions.

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