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MALDI-MS at the Ingenieurschule Burgdorf: The Technique, Some Applications and Expected Benefits for the Education in Modern Analytical Chemistry

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Abstract. Analytical chemistry is changing rapidly, new methods are developed and introduced in the analytical laboratories. Parallel to this progress, the chemical industry is undergoing pronounced changes: it is focusing more and more on the life sciences. Consequently, the importance of analytical biochemistry is increasing.
The educational system has to keep pace with these developments and has to integrate the new methods, which are or will be tools in analytical laboratories, into the analytical chemistry courses.
Therefore, we were looking for an appropriate method which would allow us to face these challenges in analytical education at the Ingenieurschule (college of engineering) Burgdorf. With the recent acquisition of a matrix-assisted laser desorption mass spectrometer (MALDI-MS), a new analytical technique which has the potential to fulfill our requirements can be offered to the chemistry students.
This contribution presents some analytical applications such as the sequence analysis of a modified oligonucleotide, the characterization of a non-covalently bound antigen-antibody complex as well as the analysis of a synthetic polymer and copolymer. Projects how to implement the method in the analytical chemistry course and the expected benefits for the students are discussed.

1. Introduction: Analytical Chemistry, a Challenge to the Education

Instrumental analytical chemistry is growing rapidly: greater speed, higher efficiency, smaller systems, more strength in terms of sensitivity, automation and new applications.

Remarkable progress has occurred in the field of mass spectrometry (MS): with the development of the new soft ionization methods electrospray ionization (ESI) mass spectrometry [1] and matrix-assisted laser desorption mass spectrometry (MALDI-MS) [2][3] in combination with powerful mass separation methods such as time-of-flight mass spectrometry (TOF-MS) or quadrupole ion trap mass analyzers, a new generation of instruments is available today. These techniques are especially well suited to analyze nonvolatile, thermally labile biopolymers due to their high sensitivity and the possibility to analyze high mass compounds of up to several 100000 Da. They are also used for other analytical applications ranging from the direct analysis of insoluble pigments to the characterization of synthetic polymers.

Young chemists and chemical engineers will get in touch with these new methods and instruments. They have to become familiar with from at least a practical point of view. To provide this is the duty of the analytical chemical education at an Ingenieurschule.

Therefore, a modern educational program has to be tailored to face these challenges and implement at least some of the new analytical techniques. These should be an integral part of the chemistry courses, therefore, versatile and flexible methods are required. Taking these points into account, we decided to acquire a MALDI mass spectrometer for the chemistry department at the Ingenieurschule Burgdorf.

The following sections demonstrate the technique, some typical applications and expected benefits of MALDI mass spectrometry for the education.

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2. The Method

The main parts of a typical linear MALDI-TOF mass spectrometer are shown in *Fig. 1*. The instrument consists of the ionizing pulsed UV or IR laser, the crystalline matrix with embedded analyte molecules and the mass analyzer. Different types of mass analyzers can be applied. In combination with the pulsed ion production, the time-of-flight mass spectrometer (TOF-MS) offers high transmission, the generation of complete mass spectra and no theoretical upper mass limit. It is, therefore, often used in MALDI instruments.

The samples are dissolved in an appropriate solvent (*ca.* 10^{-3}–10^{-5} M) and mixed with the matrix solution at a ratio of approx. 1: 1000. The matrix is an organic compound of low molecular weight which absorbs strongly at the laser emission wavelength. Approximately 1 µl of the mixture is brought onto a probe tip, the solvents are rapidly evaporated and the crystalline matrix is introduced into the vacuum system. A short laser pulse, focused onto the matrix, desorbs analyte and matrix molecules into the gas phase, where analyte ions are formed through protonation/deprotonation and/or cationization.

Recently, a promising model was proposed which allows to explain the role of the matrix and specific features of MALDI mass spectra [4].

The method was well accepted by the scientific community and has almost immediately replaced the so far successful 252-Cf plasma desorption mass spectrometry [5]. The success has further triggered the introduction of several commercial instruments. However, the method was impaired by the inherently low resolution of the most often used linear time-of-flight mass spectrometer. The recent development and implementation of the delayed ion extraction (DE) technique employing high accelerating voltages to correct for the initial velocities of the ions, has improved the resolution and the quality of the MALDI-TOF spectra considerably [6].

Education: How to operate a MALDI instrument can be learned by students within a short time. However, the quality of the

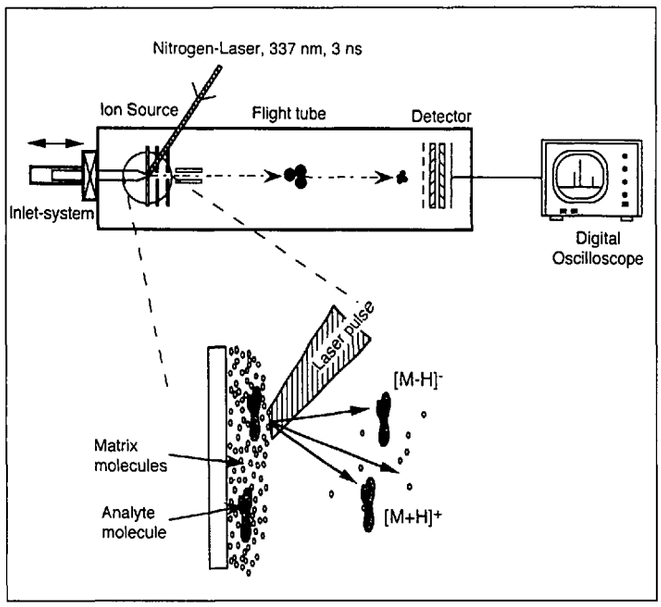

Fig. 1. *Experimental setup of a linear MALDI-TOF MS with a schematic representation of the one-step laser desorption/ionization process*

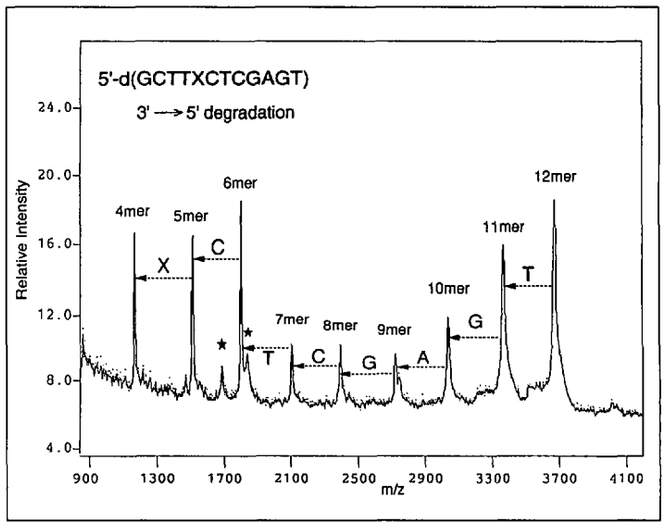

Fig. 2. *Negative ion MALDI mass spectrum of a partial digest of a modified oligonucleotide (12-mer) by Snake Venom Phosphodiesterase* [7]. The oligonucleotide carries a modified nucleoside in position 5. The peaks marked with asteriks represent doubly charged ions of '12-mer' and '11-mer'.

spectra is highly dependent on the operators skills to choose the appropriate matrix and to control the crystallization.

Although the mass spectra can be relatively uncomplicated, their interpretation by inexperienced users can lead to ambiguous results. Therefore, interpretation of the spectra has to be done under the supervision of an experienced person and parallel lectures on mass spectrometry should be provided.

3. Bioanalytical Applications

3.1. *An Example of Structure Analysis: Oligonucleotides*

The modern mass spectrometric methods allow the direct mass analysis on complex mixtures such as peptide or oligonucleotide fragments resulting from enzymatic digestions. This approach leads to sequence information without the need of amino acid or nucleotide analysis.

As an example, *Fig. 2* shows the digest of a modified 12-mer oligonucleotide with Snake Venom Phosphodiesterase (SVP) and subsequent analysis by MALDI-MS [7].

Due to the fact that the enzyme Snake Venom Phosphodiesterase (SVP), a 3'-exonuclease, cleaves the oligonucleotide only from the 3'-end, the cleaved nucleotides can be determined according to the mass difference between adjacent peaks. The oligonucleotide sequence can be directly read from the mass spectrum in the correct order. Five possible mass differences are expected, corresponding to the nucleotides pdA ('A'), pdC ('C'), pdG ('G'), pdT ('T') and the modified nucleoside 'X'. To obtain a full set of fragments, aliquots have to be taken at certain time intervals during the ongoing digestion.

Education: The determination of the molecular masses of biopolymers, intact or fragmented by enzymatic digestion, is a major target. The obtained results can be directly compared with standard methods such as polyacrylamide gel electrophoresis (SDS-PAGE).

Besides biochemical techniques, biopolymer analysis introduces coupled (hyphenated) methods in the course as a welcome side effect. *Off-line* coupling is so far the only practical way to interface liquid chromatography (LC) with MALDI-MS. The recent development of novel two phase matrices (particles in liquid) gives new hope for *on-line* coupling of LC with MALDI-MS [8]. *Off-line* coupling offers the advantages to be technically uncomplicated and to allow the combination of various methods: LC/MS coupling, gel permeation chromatography/MS coupling (GPC/MS) or thin layer chromatography-MS (TLC/MS). The students could test these combinations and notice the advantages of these hyphenated techniques in terms of analysis speed, cost, throughput, and amount of obtainable information.

3.2. *Analysis of Non-covalently Bound Complexes*

Interactions between biopolymers play an important role in numerous biochemical processes. The analysis of non-covalently bound complexes is a difficult problem for mass spectrometry because the complexes easily dissociate.

Several publications show that ESI-MS can be successfully applied to the labile non-covalently bound compounds of protein-protein as well as protein-oligonucleotide complexes [9]. MALDI-MS was less successfully applied, although the detection of intact non-covalently bound complexes were also reported [10][11].

An especially important class are antigen molecules non-covalently bound to a monoclonal antibody. The high masses of these complexes (*ca.* 150000 Da) resemble an additional challenge for the mass spectrometric analysis.

Fig. 3 shows the mass spectrum of a native monoclonal antibody and its complex with antigen molecules. Although the antigen molecules were in excess, not more than two molecules are bound per antibody. This is an indication that the binding is specific and the association is not or only slightly fragmented by the mass spectrometric analysis. The complexes could only be detected with the 2,6-dihydroxyacetophenone matrix. With the well-known matrices sinapinic acid or 2,5-dihydroxybenzoic acid no complexes were detectable. These matrices seem to promote complete dissociation of the complexes.

Education: The students are given the possibility to study non-covalent interactions directly with techniques that are manageable even in the laboratory of an Ingenieurschule.

In conclusion, MALDI-MS applied to different problems in bioanalysis is expected to yield manifold benefits for the students. They get the possibility to

- become acquainted with different bioanalytical methods,
- learn how to work with sensitive and labile biological samples,
- carry out biopolymer sequence analysis,
- study enzyme kinetics,
- test and use hyphenated techniques,
- operate the mass spectrometer.

4. Analysis of Synthetic Polymers

The conventional methods for polymer analysis are mostly averaging methods which can provide the molecular weight distributions but no information about the chemical structures. One of the classical methods to analyze polymers, gel permeation chromatography (GPC), allows to determine the molecular mass distributions of polymers. To calibrate GPC, well characterized external or internal standards should be used. But often, no appropriate standards are available.

Mass spectrometry, on the other hand, gives absolute molecular masses at a superior resolution. This allows to obtain discrete oligomeric information, determine the molecular mass distributions and characterize products and by-products by their molecular masses.

Until recently, mass spectrometry has traditionally been of only minor importance in the field of synthetic polymer analysis. Often, the molecular mass range was far in excess of what was accessible by MS or the low volatility and thermal lability of the polymers impaired the quality of the spectra. Nevertheless, field desorption (FD) coupled with magnetic sector MS or pyrolysis-MS have been used to characterize polymers.

With the development of MALDI-MS, the applicability of MS to polymers has improved. The technique can provide important chemical structure and molecular weight information of synthetic polymers and copolymers in a broad mass range. The main limitation of the method is a not well understood systematic shift of the molecular mass distributions towards lower masses for polydispersities $M_w/M_n > 1.1$ [12][13]. To circumvent this problem, the mass determination of narrow GPC fractions by MALDI-MS was proposed [14].

4.1. *Homopolymer Analysis*

The example shown in *Fig. 4*, illustrates the obtainable information about a homopolymer by mass spectrometry.

The mass M of a homopolymer, consisting of n repeat units (monomers) of mass M_m and the mass END of the end groups, is given by:

$$M = END + n \cdot M_m$$

By analyzing the masses obtained for the different oligomers in *Fig. 4*, the following information about the polymer could be obtained:

- The average mass M_m of the polymer repeat unit is 104.0 Da.
- The sample is a homopolymer (no further monomer units can be identified).
- The ion signal ($[M + Ag]^+$) with the highest intensity can be detected at mass 2246 Da. The corresponding oligomer consists of $n = 20$ monomer units (rounded, n must be an integer).
- The mass END of the end groups can then be calculated to 58.1 Da.
- The molecular mass distributions M_n, M_w and the polydispersity can be easily calculated.
- No by-products can be detected (the second detectable ion signal distribution origins from $[M_i + Na]^+$ ions).

4.2. *Copolymer Analysis*

In contrast with the relatively simple mass spectrum of the homopolymer in *Fig. 4*, a complex ion signal distribution is often obtained from copolymers as shown in *Fig. 5, a*. The mass spectrum is difficult to interpret since no ion signal sequence can be identified. To extract compositional information from copolymer mass spectra in spite of their complexity, several numerical approaches have been published [15][16]. They are rather complicated or need the knowledge of reaction constants involved in the polymerization.

A simpler numerical approach for the computer-assisted interpretation of copolymer spectra has been developed [17]: A simulation is used to generate a random copolymer mass spectrum. The program needs the masses of the different monomer units and their concentration ratio at the reaction start as input. These values are usually known from synthesis. The copol-

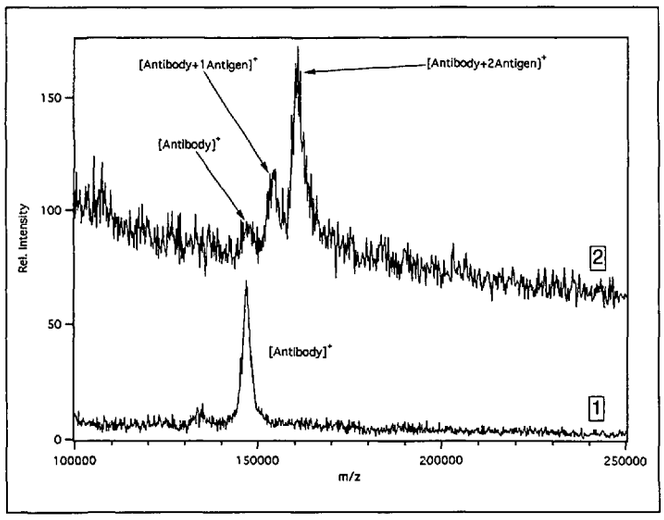

Fig. 3. *Positive ion MALDI mass spectrum of a non-covalently bound complex.* 1: Mass spectrum of a monoclonal antibody. Matrix: 0.1M 2,6-dihydroxyacetophenone in water. 2: MALDI mass spectrum of a solution of a monoclonal antibody and an antigenic peptide in excess ($c_{antigen} = 10 \cdot c_{antibody}$). Matrix: 0.1M 2,6-dihydroxyacetophenone in water. Measurements were made on a 1.7 m linear MALDI-TOF (N_2-laser, 337 nm, 3 ns).

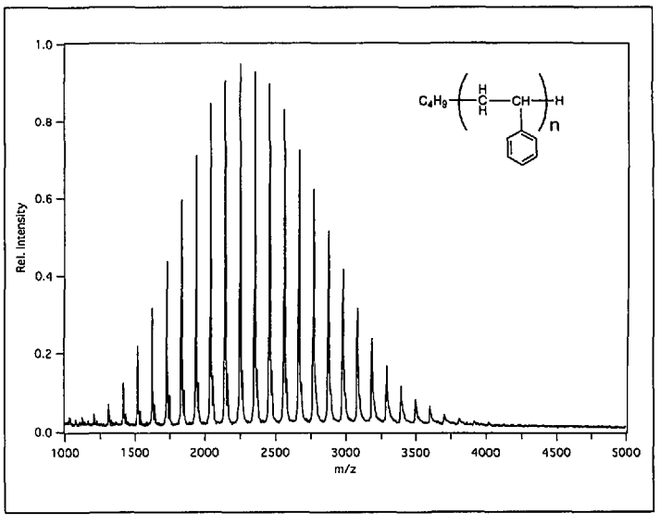

Fig. 4. *MALDI mass spectrum of polystyrene 2000.* The ions were detected as $[M_i + Ag]^+$. Low intensity ion signals of sodium adducts $[M_i + Na]^+$ can also be detected. Matrix: 0.1M 2,5-dihydroxybenzoic acid in tetrahydrofuran. Ag-salt was added. Measurement was made on a 1.7 m linear MALDI-TOF (N_2-laser, 337 nm, 3 ns).

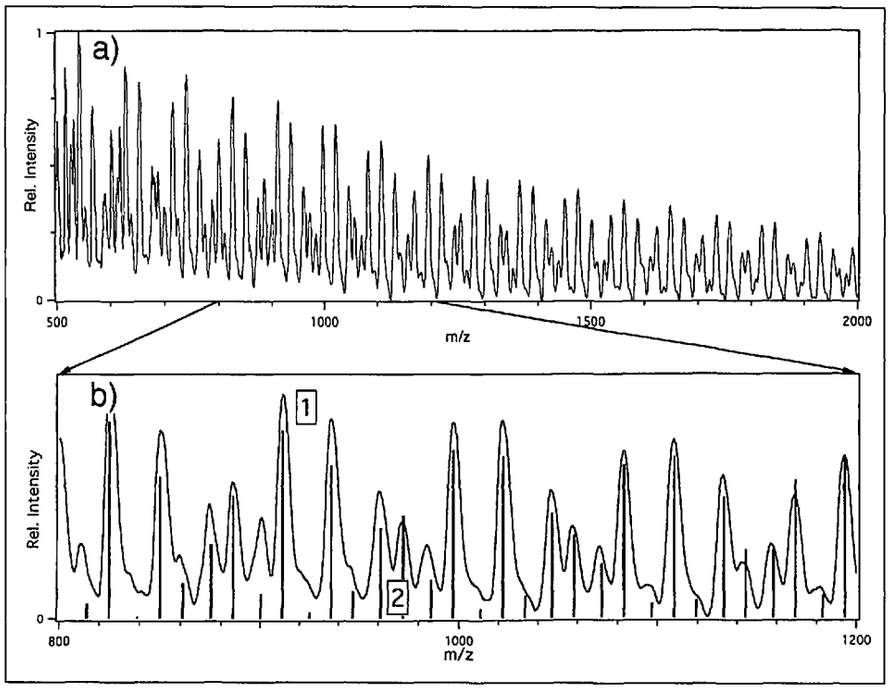

Fig. 5. *MALDI mass spectrum of a copolymer compared with a numerical simulation* [17]. *a*) Positive ion mass spectrum. Matrix: 2,5-dihydroxybenzoic acid in tetrahydrofuran. *b*) 1: MALDI mass spectrum in the mass range from 800 to 1200 Da. 2: Numerical simulation with M_1 = 111.1 Da, M_2 = 86.1 Da, ratio of concentration M_1 to M_2 = 1:3, 4000 calculations.

ymer molecules are then numerically generated by successively adding the monomer units. These units are chosen with a probability equal to their concentration ratio. By doing this for different numbers of monomers, the masses of all possible oligomers are obtained. For each newly generated mass, a corresponding artificial 'ion signal intensity' is set to 1. If the mass already exists, the corresponding 'ion signal intensity' is increased by 1. The calculations have to be repeated several thousand times to obtain average values of the ion signal intensities.

The result of such a simulation is represented in *Fig. 5, b* by a stickplot. It was known that the copolymer was polymerized of two specific monomer units with masses M_1 = 111.1 Da and M_2 = 86.1 Da. The stickplot fits the experimental data extremely well, especially if one takes into account the simplicity of the numerical simulation. The following information can be immediately deduced:

- The sample is a random copolymer.
- The best fit is obtained with two monomer units.
- The mass of the end groups can be determined.
- No or only low fragmentation induced by the analysis occurs.
- Discrimination effects in the mass spectrum can be ruled out.

The detected ion signals should thus reflect the true composition of the sample (except for the ion signal distribution over the entire mass range which cannot be predicted by this simple simulation).

Education: The visualizing effect of the mass spectra allows the students to directly observe the structure of polymers and copolymers. This is an advantage of mass spectrometry which cannot be overestimated.

In the field of polymer and copolymer analysis, an integrated course in analytical and organic chemistry is possible: synthesis of a certain polymer and subsequent analysis as part of the analytical course. As an interesting project, the coupling of GPC with MS could be studied. The calibration of GPC could thus be provided by mass spectrometry and the systematic low-mass shift of the molecular mass distributions in MALDI spectra for higher polydispersities could be circumvented [14]. The GPC/MALDI combination represents an open field for new applications to which the students of an Ingenieurschule could add valuable scientific contributions.

5. Conclusion

With the mass spectrometric MALDI method, the Ingenieurschule Burgdorf possesses a modern analytical tool with numerous benefits for the education in analytical and general chemistry.

We believe, the challenge to catch up with the rapid developments in analytical chemistry can be responded with the integration of new technologies and concepts in the analytical chemistry course. The students should thus be better prepared and armed with knowledge to work in industry and to undertake their responsibility as future decision makers. And last but not least, they have the opportunity to get in touch with a fascinating field of modern instrumental analysis.

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