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Laser-Assisted Mass Spectrometry

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Abstract. The wide range of uses for laser-assisted mass spectrometry is presented and illustrated with examples. In contrast to the practical aspects of these methods, knowledge about desorption and ion formation mechanisms in laser MS is lagging behind. Our group's efforts to unravel these mechanisms are also discussed.

My research group, at the ETH-Zürich since 1995, is active in analytical chemistry, using lasers, mass spectrometers, and optical spectroscopy as the main tools. Our main strength, I believe, is that the research is neither purely problem-oriented nor purely method-oriented; we are always striving to balance basic and mechanistic research with instrument and methods development, and with applications in a variety of fields, such as environmental science, materials science, catalysis, biological, and medical problems. We also have a wide variety of modern analytical technology available, ranging from X-ray photoeleoctron spectroscopy to mass spectrometry to high resolution laser spectroscopy. Often, researchers investigating a fundamental problem and their colleagues exploring applications are working in the same laboratory and may even use the same equipment. This creates a good basis for cross-fertilization between projects, stimulates transfer of basic research to applications, and *vice versa* encourages problems encountered in practical applications to be studied in-depth.

One of our goals is to gain chemical information from areas with dimensions much smaller than 1 μm. While this problem has been largely solved for elemental analysis (using *Auger* microscopy, *e.g.*) it remains a difficult challenge for *molecular* analysis. Our approach to meet this challenge is to develop scanning near-field optical microscopy (SNOM) for molecular analysis on a nanometer scale [1]. SNOM is a methodology that has mainly grown in the physics community but is now ripe for applications in chemical analysis. We either use SNOM probes for 'nanosampling', or spectroscopically analyze the emitted fluorescence or *Raman* scattered light from a sample illuminated in a SNOM. More detailed information about this can be found in the article by *Pohl et al.* in this issue [2].

Most of the work of our research group focuses on laser mass spectrometry, which is also the topic of this article. Lasers have been used to enhance the capability of mass spectrometry since the late 1960s [3]. In the early days, laser methods were mostly used for small spot elemental analysis of nonvolatile materials. The group of *Kistemaker* was the first to apply laser desorption mass spectrometry (LDMS) to organic materials. I consider the detection of oligosaccharides such as digoxin (*m/z* 780) and digitonin (*m/z* 1228) by LDMS as milestones in this field [4]. However, the mass range was never extended much beyond 1000 Da. This mass limit was not broken until the advent of MALDI mass spectrometry in the late 1980s.

Matrix-Assisted Laser Desorption/ Ionization Mass Spectrometry

Matrix-assisted laser desorption/ionization (MALDI) was introduced by *Hillenkamp* and coworkers [5] and almost simultaneously, by *Tanaka et al.* [6]. MALDI allows intact desorption and ionization of complex molecules up to several 100000 Da; it is now one of the most widely used modern mass spectrometric methods [7]. Ion production is initiated by a laser pulse, which also means that time-of-flight (TOF) mass spectrometers are the natural choice for mass analysis. TOFs are easy to construct and exhibit a high ion throughput, and therefore, high sensitivity. In MALDI, the sample is embedded in an excess of a solid organic matrix, which, upon laser bombardement, assists in the volatilization and ionization of the analyte molecules. Proper choice of the matrix is crucial since it has to fulfill several requirements: *i*) isolation of the analyte (*i.e.*, co-crystallization with the analyte molecules); *ii*) absorption of the laser pulse; *iii*) it has to be vacuum stable yet sublimate rapidly upon laser irradiation; and *iv*) the matrix assists in the ionization of the analyte molecules in processes that are not fully understood yet.

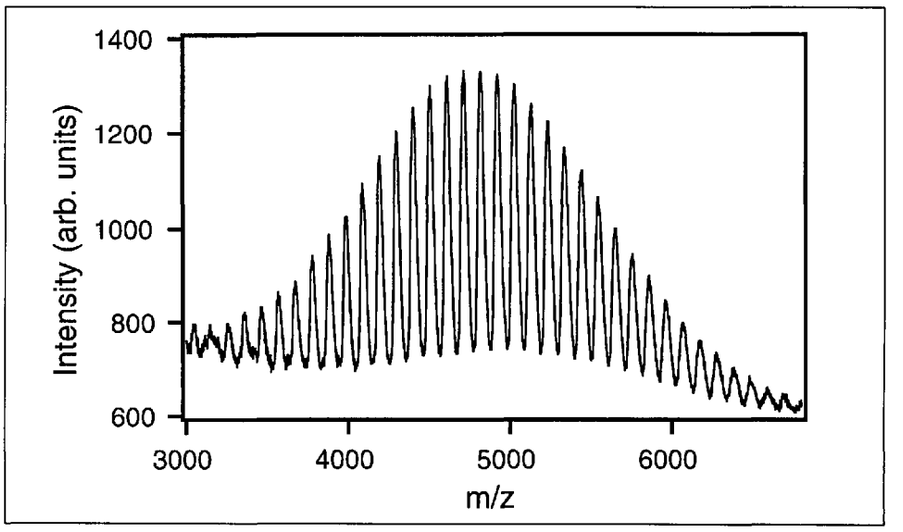

Fig. 1. *MALDI Mass spectrum of polystyrene 5000.* For sample preparation, a molar ratio of 1:114:5.7 of PS:matrix (dithranol):cationizing agent ($Cu(TFA)_2 \cdot H_2O$) was used. The experimentally determined mass difference between adjacent peaks is 104.07 Da (calc. value 104.15 Da). After subtraction of the mass of copper and an integer multiple of the repeat unit from each oligomer mass, a rest mass of 69.17 Da remains, consistent with a C_5H_9 end group, *e.g.*, cyclopentane (calc. mass 69.13 Da).

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MALDI is now well established for the mass spectrometric analysis of biopolymers such as peptides, proteins, oligonucleotides, and oligosaccharides. Synthetic polymers are also easily analyzed by MALDI-MS. *Fig. 1* shows a MALDI mass spectrum of a polystyrene sample. Important information can be directly obtained from such spectra, such as the mass of the repetition unit and information about end groups (for details, see legend to *Fig. 1*). Other properties, like the average molecular weight, average chain length, and the polydispersity can be calculated from this data. Note that MALDI-MS data of synthetic polymers are much more precise and contain much more information than analyses done with more conventional methods, *e.g.*, gel permeation chromatography or light scattering.

The mechanism of ion formation in MALDI-MS is still poorly understood. Our group has a major research effort aimed at a better understanding of this, with the ultimate goal of achieving much higher MALDI ion yields (higher sensitivity) and gaining complete control over the process. Models of MALDI ion formation include gas-phase ion-molecule reactions in the desorption plume [8], the desorption of pre-formed ions from the solid MALDI sample [9][10], charge separation induced by exciton collision processes [11], and other mechanisms [12]. To continue with the theme of synthetic polymers, consider polystyrene: like other apolar polymers it is a poor proton acceptor or donor, and is only amendable to MALDI-MS after cationization with metal ions. Silver or copper have been successfully used for the cationization of polystyrene. In principle, one could imagine desorption of pre-formed π-complexes of the cations with the polystyrene. We have recently shown that this is probably not the case. Using complementary analytical methods such as UV/VIS and IR spectroscopy, we found that such complexes were essentially absent in the solid MALDI sample, and must, therefore, form in the gas phase after desorption [10]. An interesting observation in these studies was that despite cationization with divalent cations, only singly charged peaks were seen in the mass spectra. However, it was unclear whether this was due to reduction of the copper ion to Cu^{I} or due to proton loss of the polystyrene after cationization with Cu^{II}. This issue was resolved by studying the polystyrene pentamer at much higher resolution ($M/\Delta M \approx 11\,000$) in a *Fourier*-transform ion cyclotron resonance mass spectrometer (*Fig*. 2). It was found that copper reduction was the reason for this observation (for details, see legend to *Fig. 2*).

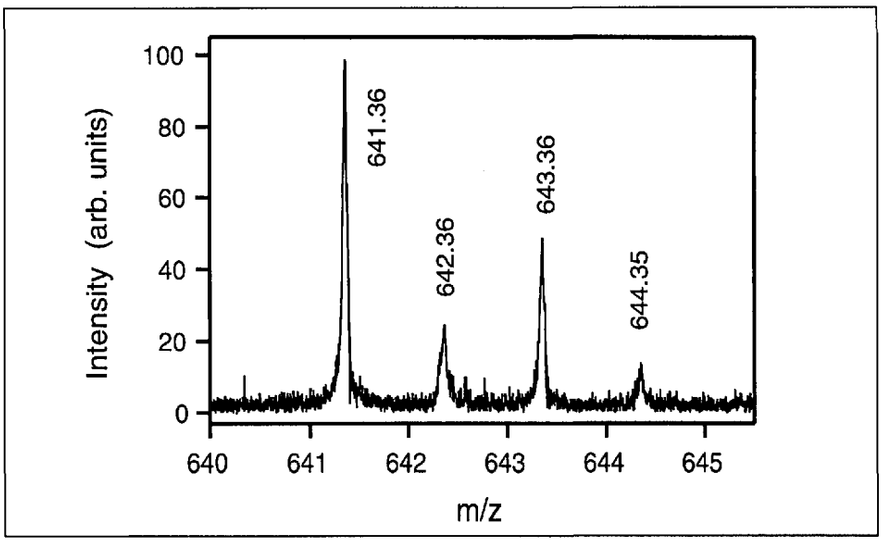

Fig. 2. *FT-ICR Mass spectrum of polystyrene pentamer* ($C_{44}H_{50}$) *cationized with a copper(II) salt.* The sample preparation was identical to *Fig. 1*. Only singly charged ions are observed, despite of the use of copper(II) for cationization. The high resolution allows to clearly distinguish between a deprotonated species containing Cu^{II}, $[C_{44}H_{50}–Cu^{II} – H]^+$ (calc. *m/z* 640.31, not observed), and a species cationized with a reduced Cu^{I} ion, $[C_{44}H_{50}–Cu^{I}]^+$ (calc. *m/z* 641.32), which is the base peak. Other peaks can be assigned to $[C_{43}{}^{13}CH_{50}–Cu]^+$ (calc. *m/z* 642.32), $[C_{44}H_{50}–^{65}Cu]^+$ (calc. *m/z* 643.32), and $[C_{43}{}^{13}CH_{50}–^{65}Cu]^+$ (calc. *m/z* 644.32). The average difference between the calculated and the experimentally determined masses was only 0.04 Da.

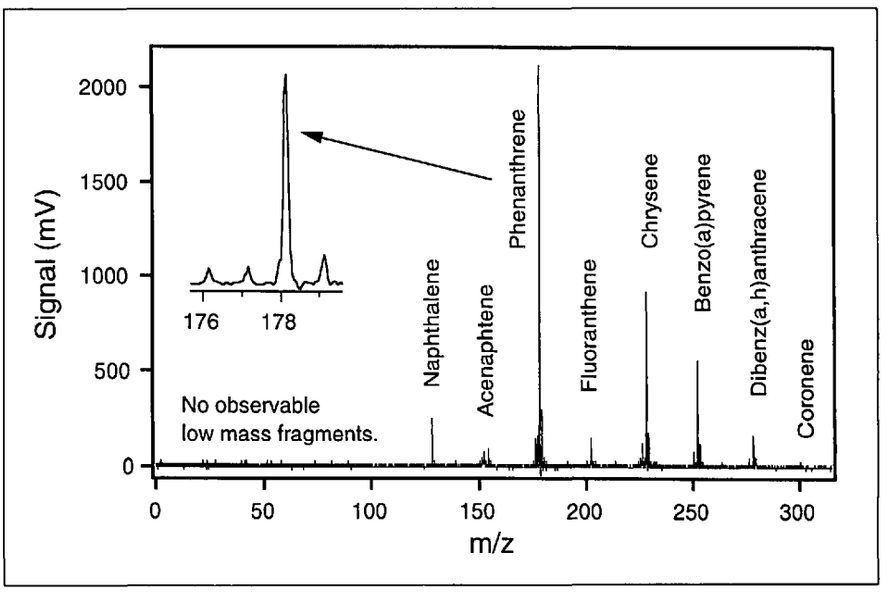

Fig. 3. *L2MS Spectrum* (single shot measurement) *of polycyclic aromatic hydrocarbons embedded in a 50 μm thick polyvinyl chloride membrane, dyed with methyl red.* Ionization wavelength 250 nm, pules energy 40 μJ/shot, ablation fluence *ca.* 6 J/cm^2, mass resolution 1200. Amounts varying between 100 fmol (coronene) and 550 fmol (naphthalene) are desorbed per shot from a 180 × 135 μm elliptical spot. The signal intensities depend both on the concentration and on the UV-absorption characteristics of the individual compounds.

In situ Chemical Analysis by Two-Step Laser Mass Spectrometry

A disadvantage of MALDI-MS is that it requires co-crystallization of the sample with the matrix from a solution. Sometimes, this is impossible due to restrictions in solubility. It also implies that direct, *in situ* analyses are generally not possible by MALDI. In this situation, another method known as Two-Step Laser Mass Spectrometry (L2MS) can be applied. L2MS uses laser induced thermal desorption from a surface by an IR laser pulse, followed by resonant multiphoton ionization by an UV laser pulse and time-of-flight mass analy-

sis [13]. Its greatest advantage is the highly efficient, optically selective, and soft photoionization step that allows selective detection of compounds in complex mixtures.

L2MS has recently received much attention when it was used by *Zare*'s group for the spatially resolved analysis of organic matter in a Mars meteorite recovered in the antarctic [14]. It can also be employed for the study of environmental samples [15][16], biolomolecules [17][18], organic thin films [19], polymer additives [20], and in many other applications (for reviews, see [21–23]).

As an illustration of the capabilities of L2MS, *Fig. 3* shows the analysis of a mixture of polycyclic aromatic hydrocarbons (PAHs) embedded in a colored polymer matrix. This example continues with the theme of polymer analysis, but this time the target compounds are trace constituents present at very low levels in the polymer. PAHs were chosen because of our long-standing interest in them as environmental contaminants and carcinogens. They possess excellent chromophores for resonant multiphoton ionization and can be detected with great sensitivity and selectivity. In this example, the selectivity is dramatically demonstrated: neither the dye (methyl red) nor the polymer, present at a 26000-fold excess is detected in the L2MS spectrum.

In our group, we also carry out basic research on the mechanism of laser-induced thermal desorption, the first step in L2MS. The central question is how large polar and thermally labile molecules can survive the laser desorption process intact. Using surface science tools and laser spectroscopic methods, we are studying the kinetic and internal energy distributions of test molecules after laser desorption from a variety of substrates [24]. In the course of this work, we also developed a method based on blackbody radiation for noncontact temperature measurement of laser heated surfaces with < 10 ns time resolution [25].

Our most recent mechanistic results indicate that the laser desorption process, even with picosecond laser pulses, can be fully described by thermal equilibrium kinetics [26]. No 'bottlenecks' for energy flow [27] or nonequilibrium during the laser desorption event, as believed by many, seem to be invoked. The final goal of this work will be to design optimum substrates for intact laser desorption of any kind of molecule, and to tranfer this know-how to practical L2MS applications. In one case, we have shown that this is possible: silylation of silica substrates reduced the internal temperature of laser-desorbed tryptophan by a factor of three, as apparent from dramatically reduced fragmentation [28]. This was due to the elimination of hydrogen bonding between silanol groups on silica and the adsorbed tryptophan.

The area of laser mass spectrometry continues to provide challenges both in application oriented and in fundamental research. We believe that our group is well positioned to meet these challenges. Particularly exciting to all of us is how methods from many different fields, ranging from biology to physics can be combined for optimum problem solving strategies.

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