

Mass Spectrometry of Molecular Adsorbates Using Laser Desorption/Laser Multiphoton Ionization

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Current and potential applications of a new mass spectrometric technique for the analysis of nonvolatile and thermally labile substances are reviewed. The method is compared to other approaches and its advantages are discussed.

For the purposes of chemical analysis, gas chromatography combined with mass spectrometry (GC-MS) is often the method of choice for volatile compounds.¹⁾ Here one relies on GC to separate components in a complex mixture and MS to identify them in terms of parent mass and daughter fragments. The GC-MS combination allows quantitation over a wide range of concentrations down to the parts per billion level. For nonvolatile compounds or compounds which decompose upon heating, one generally relies on liquid chromatography (LC) or electrophoresis. However, these techniques are not easily interfaced to mass spectrometers so that they generally do not partake of the identification powers of GC-MS. One effort to overcome this difficulty is to apply mass spectrometry to the analysis of molecular surface overlayers. Often this is accomplished by a one-step process in which molecules are ionized and ejected from the surface by bombardment with energetic particles, such as by electrons (ESD),²⁾ ions (SIMS),³⁾ fast neutral atoms (FAB),⁴⁾ fission fragments (plasma desorption),^{5–7)} and photons (laser desorption).^{8–14)} Compounds of major interest include peptides, proteins, polynucleotides, porphyrins, oligosaccharides, and biomolecules having strong acidic, basic, or polar side groups. However, it is necessary to understand in detail the ejection and ionization mechanism at the surface in order to relate the observed mass spectrum to the nature of the molecular adsorbate. Often this is obscured by matrix-dependent ionization as well as extensive fragmentation. We are exploring an alternative approach in which the desorption step is separated in time and in space from the ionization step.^{15,16)} This method has first been applied to the trace detection of atoms from surfaces,^{17–22)} then molecules from surfaces.^{23–26)} Many variations are possible, but our preferred embodiment of this method involves an infrared laser to desorb essentially intact molecules from a substrate opaque to this wavelength followed, after a suitable delay, by an ultraviolet laser to cause resonance-enhanced multiphoton ionization (REMPI) of those species absorbing the second wavelength. In comparison with other mass spectrometric methods, the two-step laser desorption/laser multiphoton

ionization method has the advantages:

- short analysis time
- high sensitivity
- high selectivity in ionization
- no matrix ionization effects
- simple mass spectrum often dominated by the parent ion peak
- large dynamic range of quantitation

In what follows we discuss these features and review our present efforts to develop this new mass spectrometric technique.

Two-Step Laser Desorption/Multiphoton Ionization Mass Spectrometry

A. Laser Desorption. Our two-step methodology has been described previously.^{15,16)} As a first step (Fig. 1a), a CO₂ laser pulse (10.6 μm ; ca. 10 mJ pulse⁻¹; 10 μs pulse width; 10 Hz repetition rate) is directed onto a thin film of molecular adsorbate deposited on the surface of a suitable solid substrate, e.g., glass or machinable ceramic (MACOR). The substrate is rapidly heated and neutral molecules are desorbed from the surface (Fig. 1b).

The rate of heating induced by the CO₂ laser pulse is approximately 10⁸ K s⁻¹. This should be compared to heating rates of 10 K s⁻¹ or less, which can be obtained by traditional resistive heating. For adsorbates that undergo extensive molecular decomposition on the surface at a conventional heating rate, the rapid laser induced heating rates can favor desorption over decomposition.^{27,28)} It has been proposed²⁹⁾ that the van der Waals type bonds between the physisorbed molecules and a solid substrate may serve as a "bottleneck" for energy transfer from a rapidly heated surface to the internal degrees of freedom of the adsorbed molecules, causing internally lukewarm intact molecules to be desorbed. There is much experimental evidence to support the claim that relatively cold molecules escape from a hot surface by laser-induced thermal desorption (LITD).^{30–33)} The same type of behavior in which evaporation dominates over decomposition has also been observed for large molecules desorbing from surfaces following

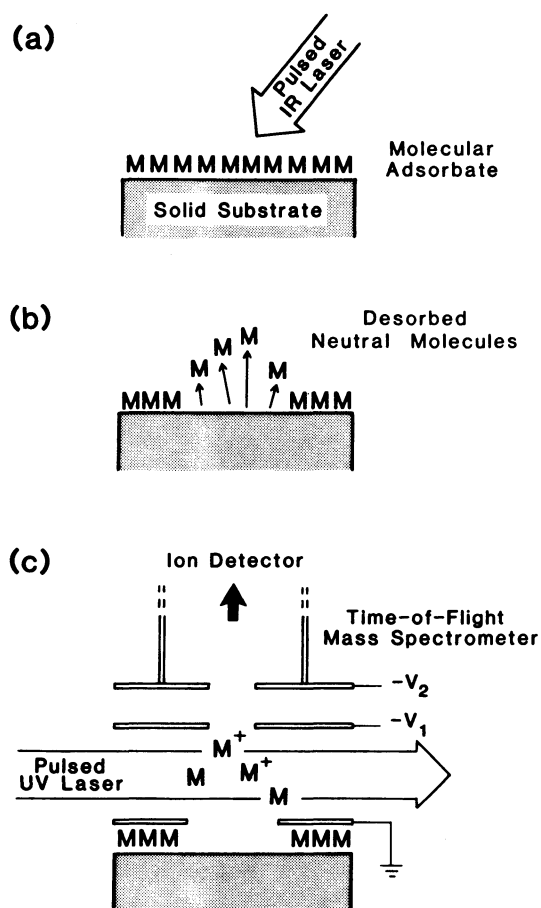


Fig. 1. Procedure for two-step laser desorption/ionization multiphoton ionization mass spectrometry: (a) a CO_2 laser pulse is directed onto a film of molecular adsorbate; (b) neutral molecules escape from the surface by rapid thermal desorption; and (c) after a suitable time delay, the fourth harmonic of a Nd:YAG laser selectively ionizes neutral molecules, and the ions are then detected by a time-of-flight mass spectrometer.

pulsed laser heating.^{8,9,15,16,26,27,34-37} Moreover, nonvolatile large molecules tend to desorb by LITD rather than by a resonant infrared absorption. For example, we have found that the parent ion signal of protoporphyrin IX dimethyl ester evaporated from a glass surface by a CO_2 laser pulse is 3×10^5 times stronger than that from a NaCl surface (which is transparent at the CO_2 laser wavelength).

B. Multiphoton Ionization. In the second step (Fig. 1c) of our methodology, desorbed molecules are ionized by 1+1 REMPI with the fourth harmonic (266 nm) of a Nd:YAG laser (ca. 1 mJ pulse^{-1} ; 10 ns pulse width; 10 Hz repetition rate). The appropriate delay (70–90 μs) between the desorbing CO_2 laser and the ionizing Nd:YAG laser pulse is chosen so that the ionizing laser pulse intercepts as many molecules as possible.

In 1+1 REMPI, one photon causes a molecule to make a transition to an electronically excited state and a second photon ionizes the excited molecule. Wavelength selectivity results from the photon energy being resonant with an intermediate state while such selectivity is lost in the direct one-photon ionization process.³⁸ REMPI has other important attributes for mass spectrometry; it can provide very efficient “soft ionization”^{15,16,34-42} in which the parent molecular ion dominates the mass spectrum. This is in sharp contrast to electron impact ionization where soft ionization can be obtained only with a significant decrease in ionization efficiency. In our experiments the Nd:YAG laser was slightly focused by a quartz lens to a 6 mm diameter spot size, resulting in a power density of about 10^6 W cm^{-2} in the ionization volume.

In contrast to direct one-step ionization of molecular adsorbates, the two-step laser methodology where the desorption and ionization processes are spatially and temporally separated allows a much larger fraction of the molecules to be converted to parent ions by optimizing separately the two laser sources. In addition, this technique avoids the variations of the ionization efficiency with surface substrate (matrix effect).

C. Ion Detection. Because the ion production is pulsed, it is natural to carry out mass analysis in a linear time-of-flight (TOF) system^{15,16,26} or in a reflectron TOF mass spectrometer.^{35-37,43} High-resolution Fourier-transform ion cyclotron resonance (FI-ICR) mass spectrometers may also be used as detectors.^{44,45}

In our setup, the ions are mass-separated in a 30 cm flight tube and detected by an electron multiplier connected to two preamplifiers which feed a transient digitizer. Our typical mass resolution with this arrangement is about 100; we are presently modifying the TOF instrument into a reflectron configuration to provide much higher mass resolution.

D. Sample Preparation. In order to achieve the quantitative analysis of molecular adsorbates, it is essential to make a homogeneous film on a solid substrate. Figure 2a shows a schematic drawing of the system for preparing a thin sample film. Application of about 200 μl of the sample solution to a glass cup provides a convenient sample size for quantitative analysis. After application of the solution, the glass cup is spun horizontally by a motor and the solution spreads out as a layer over the inner cylindrical surface. A vacuum is applied to evaporate the solvent, resulting in a dry homogeneous film with known thickness. The glass cup is then mounted onto the tip of a 1/2 inch diameter Teflon rod, directly introduced into the main vacuum chamber (ca. 10^{-7} Torr) through a gate valve without breaking high vacuum, and placed in the center of the first electrode of our TOF instrument. The direct sample introduction

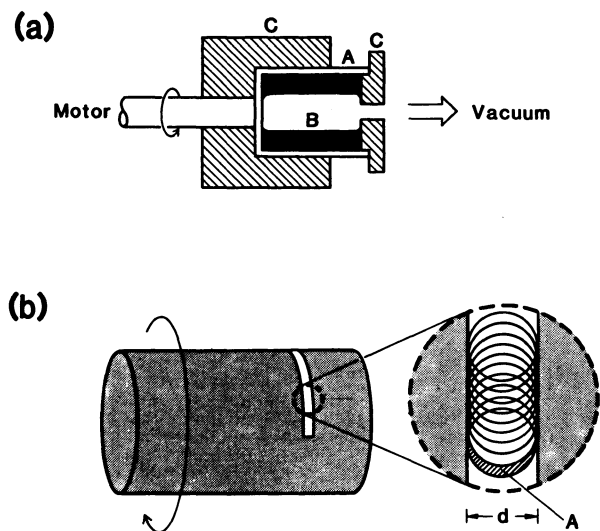


Fig. 2. (a) Apparatus for making a thin homogeneous sample film. Here A is a glass cup (12 mm \times 20 mm), B represents the sample solution layer formed as the glass cup is spun horizontally and by a motor, C is a Teflon clamp and cap assembly. (b) A CO₂ laser desorption track on the inner surface of the glass cup: d is the diameter of the desorption laser spot (ca. 1 mm) and A is the new area exposed in consecutive laser shots (ca. 5×10^{-2} mm²).

method takes about one minute, and the mass spectrum can be recorded without further delay.

Representative Applications

Figure 3 shows typical laser desorption/laser multiphoton ionization mass spectra of a purine base, a dipeptide, a steroid, and a porphyrin. Common properties of these molecules are low volatility, high polarity, and a UV chromophore allowing for 1+1 REMPI (in the form of an aromatic or π -conjugated system). The most important features of these spectra are dominant parent ion peaks in all cases. Almost complete absence of fragmentation in Fig. 3c and 3d demonstrates the desorption of intact parent molecules in the laser evaporation step as well as the soft ionization capabilities of the REMPI process. The amount of fragmentation depends on the ionization laser fluence. For adenine, e.g., almost no fragmentation occurs at lower laser fluence.¹⁶⁾ Of course, fragmentation can also be a virtue in the identification of large molecules, and controlled fragmentation is easily achieved by increasing the fluence of the laser used in the ionization step. To some extent, fragmentation also depends on the nature of the surface/adsorbate bond: For gly-phe (Fig. 3b) and other dipeptides fairly intense fragments show up in the mass spectrum.³⁴⁾ On the other hand, it is possible to observe parent ions of a decapeptide (MW=1295 amu)³⁶⁾ and of proteins up to a mass of 23000 amu

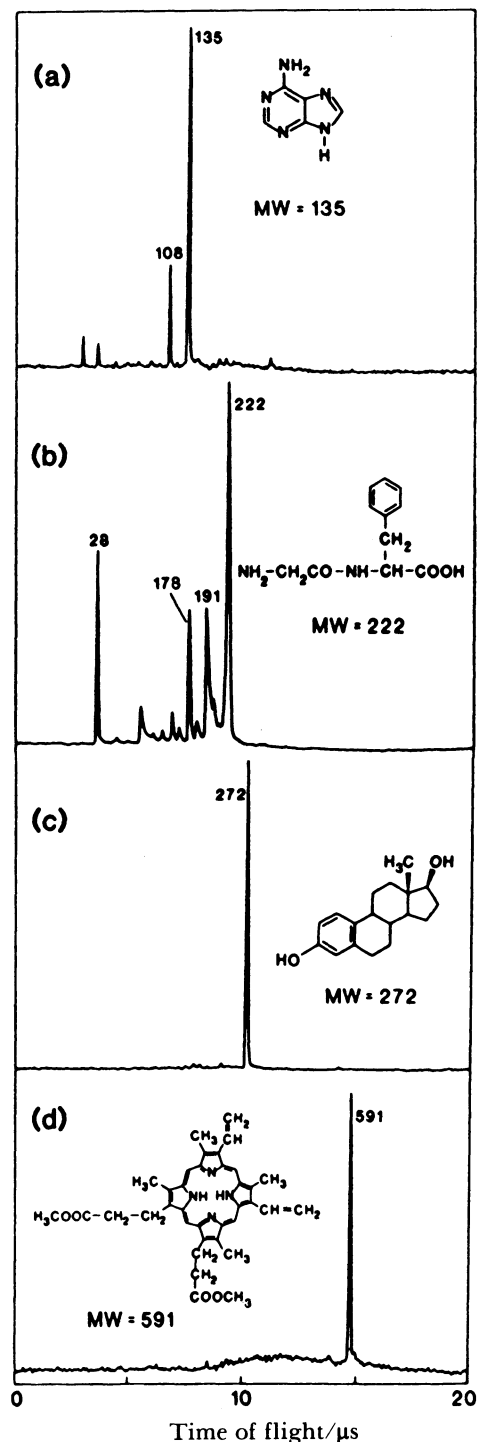


Fig. 3. Laser desorption/laser multiphoton ionization mass spectra of (a) adenine, (b) glycyl-L-phenylalanine (gly-phe), (c) β -estradiol, and (d) protoporphyrin IX dimethyl ester. Each mass spectrum represents an average over 200 laser shots.

(using ²⁵²Cf bombardment).⁶⁾ We still lack a thorough understanding of various desorption mechanisms, and the examples above indicate that we need a better theoretical description in order to optimize the two-step process.

Higher-mass compounds are also tractable with our technique, as shown in Fig. 4. These two synthetic porphyrins again exhibit single parent ion peaks at 1132 and 1232 amu, respectively. This class of compounds is readily ionized by the fourth harmonic of the Nd:YAG laser. For protoporphyrin IX dimethyl ester (Fig. 3d) we have found¹⁶⁾ a detection limit of 4×10^{-17} mol.

Dominant parent ion peaks enable us to record spectra of mixtures. Figure 5 shows an example of an equimolar mixture of 5 phenylthiohydantoin (PTH)-amino acids. These compounds are the final products of the Edman degradation method used in protein sequencing. In our original work, all the 20 PTH-amino acids, corresponding to the 20 naturally occurring amino acids could be analyzed.¹⁵⁾ We were even able to distinguish between the leucine and isoleucine isomers owing to a difference in the fragmentation pattern. An example of a different sort is provided by the analysis of a meteorite sample.⁴⁶⁾ Figure 6 shows a mass spectrum of material from Murchison, a meteorite rich in organic carbon compounds.⁴⁷⁾ By spiking the sample with various

standards, it was possible to identify a series of polynuclear aromatic compounds in the mass range from 120–300 amu, which is consistent with previous results.⁴⁸⁾ Please note by its conspicuous absence the lack of porphyrins in this meteorite sample. This is now an accepted fact, but was the subject of a scientific debate for a long time.⁴⁹⁾

A particularly useful feature of our methodology is the linear quantitation we have achieved using the sampling technique described above. From experiments, where we exposed the same spot to the

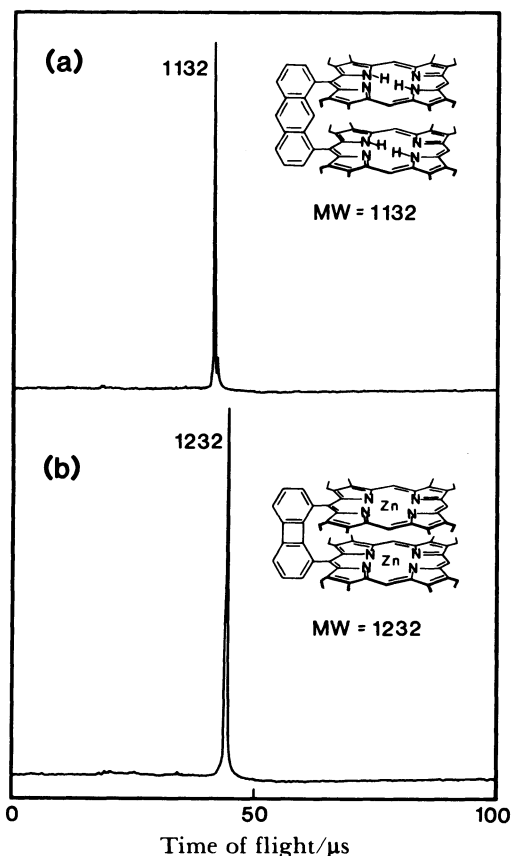


Fig. 4. Laser desorption/laser multiphoton ionization TOF mass spectra of (a) H₄(DPA) and (b) Zn₂(DPB).

DPA=1,8-bis(3,7,13,17-tetraethyl-2,8,12,18-tetramethyl-5-porphyrinyl)anthracene; DPB=1,8-bis(....)biphenylene.

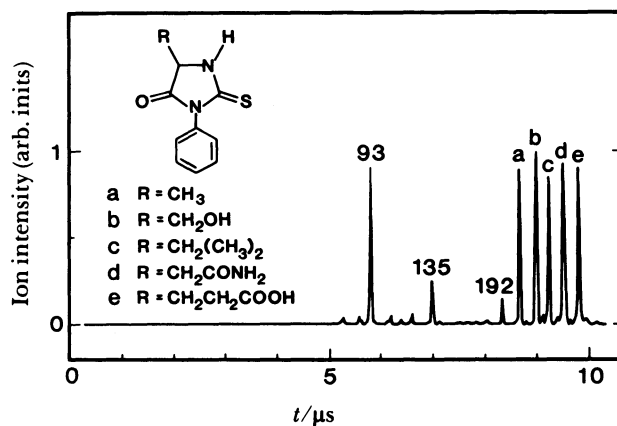


Fig. 5. A mixture of equimolar amounts of (a) PTH-alanine, (b) PTH-serine, (c) PTH-valine, (d) PTH-asparagine, and (e) PTH-glutamic acid analyzed by laser desorption/laser multiphoton ionization mass spectrometry. The peaks labeled a–e are the parent ions; some fragments of the compounds in the mixture superimpose in the lower mass region and give rise to the signals at 93, 135, and 192 amu.

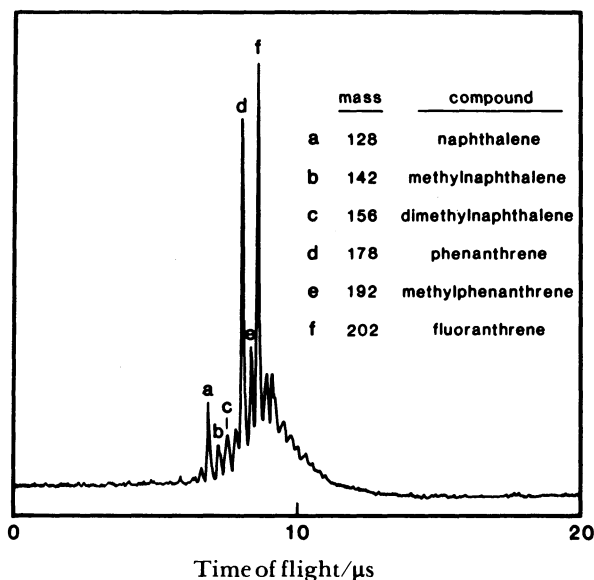


Fig. 6. Laser desorption/laser multiphoton ionization mass spectrum obtained from 1 μg of the Murchison meteorite.

desorption laser twice, we found that the desorption of the adsorbate is essentially complete after the first shot. Spectra taken for quantitative studies represent an average over many shots. Here we rotate the sample and expose a small surface area, A , to each new laser shot. This area can be calculated from a knowledge of the repetition rate of the desorption laser, the rotation speed of the sample, and the measured laser track width (cf. Fig. 2b). The amount of adsorbate which is removed per laser shot, n , is then calculated by

$$n = n_{\text{tot}} \cdot (A/A_{\text{tot}})$$

where n_{tot} is the total of amount of adsorbate deposited on A_{tot} , the total area covered. By monitoring how the signal height of the parent ion peak varies with adsorbate concentration, we found a linear dependence of n over a range of 5 orders of magnitude, as shown in Fig. 7.¹⁶ The amount of adsorbate desorbed per laser shot ranges from nanomoles (100 monolayers) to femtomoles (10^{-4} monolayers).

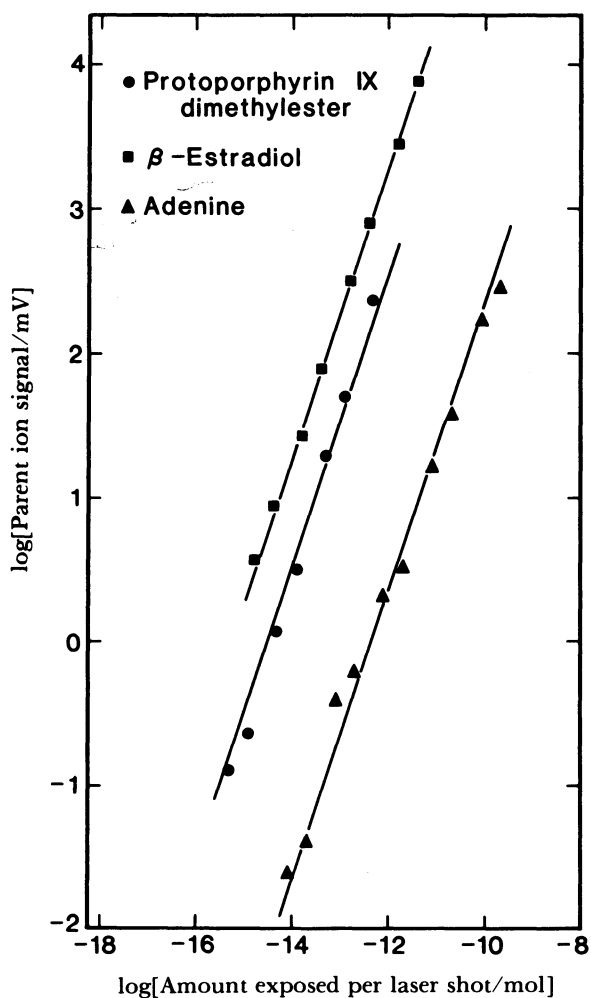


Fig. 7. Quantitation: Parent ion signal of protoporphyrin IX dimethyl ester, β -estradiol, and adenine vs. surface concentration.

Concluding Remarks

Laser desorption/laser multiphoton ionization has a number of appealing advantages for the mass spectrometric analysis of molecular adsorbates: high sensitivity, controlled fragmentation, selective ionization, rapid quantitation, and negligible matrix effects. This technique is in its early infancy but the applications to large biomolecules and other thermally labile and/or nonvolatile compounds are quite encouraging. More needs to be known about the desorption mechanism involving the adsorbate/substrate interaction before this method can be routinely applied. Nevertheless, this method has particular promise for the analysis of samples of limited availability, suggesting that some of its most dramatic uses may be in the analysis of rare samples, such as, fossils, lunar soils, meteorites, cosmic dust, and other cosmogeochemical materials.

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