

RESTRICTED ENERGY TRANSFER IN LASER DESORPTION
OF HIGH MOLECULAR WEIGHT BIOMOLECULES

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Abstract

Producing ions from large molecules is of distinguished importance in mass spectrometry. In our present study we survey different laser desorption methods in view of their virtues and drawbacks in volatilization and ion generation. Laser induced thermal desorption and matrix assisted laser desorption are assessed with special emphasis to the recent breakthrough in the field ($m/z > 100,000$ ions produced by matrix assisted laser desorption). Efforts to understand and describe laser desorption and ionization are also reported. We emphasize the role of restricted energy transfer pathways as a possible explanation to the volatilization of non-degraded large molecules.

Key words: laser desorption, laser ionization, mass spectrometry, biomolecules, peptides, proteins, nucleotides, nucleosides, saccharides, energy transfer, phase transition, disintegration, fragmentation, ion formation.

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Introduction

With the growing importance of biomedical investigations in organic analysis the emphasis has been shifting to the detection and structure determination of ever larger and more complex molecules. Gas phase analytical methods in general — and mass spectrometry was not an exception — exhibited increasing handicap with the growing molecular masses, because of the involatility and instability of these materials.

The early answer to this challenge came in the seventies, in the form of the so called "soft" ionization techniques. Field desorption, chemical ionization, plasma desorption, secondary ion, electrohydrodynamic and laser desorption ion sources were developed to cope with non-volatile compounds. A thorough overview of these methods with respect to their high mass capabilities was published by Daves in 1979. There seemed to be two distinct strategies to follow: (a) optimization of volatilization conditions in terms of sample dispersion and heating rate and (b) direct ionization of molecules from a surface by external electrostatic field, particle bombardment or laser radiation. At this stage of development, however, analytical possibilities were limited to the mass range $m/z < 10,000$.

During the eighties four techniques were able to overcome this limit. ^{252}Cf plasma desorption mass spectrometry (Sundqvist and Macfarlane, 1985) became available in the $m/z < 45,000$ mass range. Fast atom bombardment — an offspring of secondary ionization with special sample preparation — reached the $m/z \approx 24,000$ region (Barber and Green, 1987). The two forerunners, however, became the electrospray ionization (Fenn et al., 1990) and matrix assisted laser desorption (Karas et al., 1989a) with $m/z = 133,000$ and $m/z = 250,000$ high mass records, respectively. The latest developments indicate capabilities for both techniques even beyond these limits (Nohmi and Fenn, 1990; Williams and Nelson, 1990).

On the other hand, one should not overemphasize the value of molecular weight determination by these methods. Several other, long established techniques are available in the same or even in broader molecular weight range. Ultracentrifugation, light scattering,

gel permeation chromatography and gel electrophoresis cover the molecular weight region from about 1,000 to about 5,000,000 (Siggia, 1968). The cost and availability of the necessary instrumentation compares favorably to the mass spectrometric equipment. When assessing the different molecular weight determination methods it is necessary to keep in mind that mass spectrometry provides directly mass to charge ratio data whereas the separation techniques measure particle mobilities and may incorporate systematic errors.

An important aspect of the analytical value of the mass spectrometric methods is their remarkably high sensitivities and low detection limits. Recent reports on electrospray ionization put sensitivities in the low pmol/ μ L and detection limits in the low fmol region for several $556 < m/z < 66,000$ peptides (Van Berkel et al., 1990). Both sensitivity and detection limits, however, are highly compound dependent. The detection limit increases with increasing molecular weight, because the ion signal is distributed over more charged states (Loo et al., 1990).

Similar, or even better results were obtained with laser desorption. The sensitivity was in the low pmol/ μ L, whereas the detection limits in the sub-pmol (Karas et al., 1990) ($10,000 < m/z < 100,000$ peptides), or in the sub-fmol region (Hahn et al., 1987) (for porphyrin derivatives).

Naturally, these numbers should be evaluated with the achievements of other techniques in mind. As an example, we quote here some results on the analytical performance of open tubular liquid chromatography. As little as 4 fmol protein was hydrolysed and derivatized resulting in approximately 25 nL volume analyte solution. Quantitative determination of 14 amino acid constituents was carried out with about 5 % error (Oates and Jorgenson, 1990). Chromatographic and immunoassay analysis are serious competitors in terms of sensitivity, with usually much lower cost of instrumentation and consequently with better availability.

Adventures to volatilize large molecules and detect minute quantities with the aid of lasers and mass spectrometry will be the topic of this review. Laser desorption mass spectrometry of nonvolatile organic molecules was surveyed by Shibanov (Shibanov 1986), but vigorous development in the field since then has changed the landscape considerably.

We will focus our attention on what the different laser desorption and ionization methods have to offer in excess of the capabilities of the more widespread analytical tools. Special emphasis will be given to the mass range and mass accuracy, to detection limits and sensitivity, to the freedom from matrix interferences and to the availability of structural information. Two types of experiments will be distinguished in separate sections: laser induced thermal desorption and matrix assisted laser desorption.

In the last section we enlist and comment on the

existing ideas and models which try to explain laser desorption of large molecules. The concepts range from rapid heating (Beuhler et al. 1974) to mechanical approaches (Williams, 1990), to restricted energy transfer (Vertes, 1990) and to expansion cooling of the laser generated plume (Vertes et al., 1989b, Nelson et al., 1989).

Laser induced thermal desorption

The idea of laser induced thermal desorption (LITD) stems from early studies of the influence of laser radiation on small molecules adsorbed on solid surfaces. If the substrate absorbs the laser radiation, it heats up on a time-scale comparable to the laser pulse length. The resulting temperature rise leads to the detachment of the adsorbed molecules.

It was realized soon that Q-switched laser pulses (durations are on the ns time-scale) may lead to sub-thermal velocity distributions of the desorbed particles (Wedler and Ruhmann, 1982). Further studies suggested that the desorption process is adiabatic, especially in the case of monolayers (Simpson and Hardy, 1986).

The possibility to desorb cold molecules from hot surfaces seemed very attractive and triggered studies on larger molecules. The conflict of volatilization versus thermal lability of these compounds was thought to be overcome (van der Peyl et al., 1982a). Calculated surface temperatures showed strong correlation with the generated ion currents. To implement the method for higher mass compounds, however, was apparently not completely successful: $m/z \approx 10,000$ seemed to be an upper limit of ion production by LITD (James and Wilkins, 1988).

Clearly, the evolution of substrate surface temperature plays a decisive role in the course of events in LITD. Temporal and spatial temperature distributions, $T(\mathbf{r}, t)$, in the substrate can be described by the heat conduction equations:

$$C(T) \frac{\partial T(\mathbf{r}, t)}{\partial t} = - \text{div} J(\mathbf{r}, t) + I(\mathbf{r}, t), \quad (1)$$

$$J(\mathbf{r}, t) = K(T) \nabla T(\mathbf{r}, t) \quad (2)$$

where $I(\mathbf{r}, t)$ is the source term describing the laser heating and $J(\mathbf{r}, t)$ is the heat flow. $C(T)$ and $K(T)$ are the specific heat and the heat conductivity, respectively, generally exhibiting considerable temperature dependence. The source term can be expressed in terms of laser irradiance, $I_0(\mathbf{r}, t)$, corrected for surface reflection:

$$I(\mathbf{r}, t) = \alpha I_0(r, t) \exp[-\alpha z] \quad (3)$$

where the light propagates along the z axis perpendicular to the substrate surface. α is the light absorption coefficient of the substrate at the laser wavelength.

The solution of the linear problem (K and C constant) for general laser intensity distributions and for the special case of a Gaussian beam was found by Lax in 1977. The non-linear problem of temperature dependent heat conductivity was also solved analytically (Lax, 1978).

Experimental results became also available to trace surface temperature rise as the consequence of a laser pulse. Resistance change of a vapor deposited platinum stripe on an insulating substrate provided time resolved information on surface heating (Zenobi et al., 1988). Comparison of the measured temperatures with analytical solutions of Eqs. (1-3) in the linear, one-dimensional case and with numerical solutions in the nonlinear case concluded in favor of the latter (Philippoz et al., 1989).

Both experiment and calculations indicated extremely high heating rates, generally in excess of 10^8 K/s. In the study of Deckert and George, 1987, surface decomposition reaction and desorption were considered to be competing first order processes. It was possible to show the existence of a crossover point of the product yield curves indicating the takeover of desorption.

Analytical application of LITD excel in particularly low detection limits. In the case of protoporphyrin IX dimethyl ester, subfemtomol quantities of the analyte provided detectable signals ($S/N=2$) (Hahn et al., 1987). The molecules were desorbed by a CO_2 laser pulse, and subsequently ionized by a frequency quadrupled Nd-YAG laser. Ion detection was carried out by a time-of-flight mass spectrometer. Linear dependence of the parent ion signal intensity with adsorbate concentration was found over a range of five orders of magnitude (Zare et al., 1988) offering remarkable quantitation possibilities.

Matrix assisted laser desorption

Another possibility to deliver the laser energy to the sample is direct light absorption. In this scheme the sample has to have high absorption coefficient at the laser wavelength. For commonly used ultra violet lasers this condition is not met for a large number of important biomolecules. Therefore, the idea of mixing these samples with a good absorber came as a possible enhancement of the method (Karas et al., 1987). Indeed, resonantly absorbing substances exhibited about an order of magnitude lower threshold irradiances (the lowest irradiance necessary for ion generation). The lower the irradiance is, the softer the ionization method can be considered.

Based on this observation, the matrix assisted laser desorption (MALD) method was introduced, essentially as a special sample preparation technique. In the early version of the experiment a dilute solution of the analyte ($\sim 10^{-5}M$) was mixed with an equal amount of a $5 \times 10^{-2}M$ aqueous solution of nicotinic acid. A droplet of this mixture was air dried on a metallic substrate and introduced into the mass spectrometer. Moderate irradiance (10^6 - 10^8 W/cm²) frequency quadrupled Nd-YAG laser pulses (266 nm, 10 ns) were used to desorb and

ionize the sample. Mass analysis was carried out by a time-of-flight mass spectrometer.

Achieving softer ionization conditions for a broader class of materials was a remarkable advance in itself. Further exciting developments came with the introduction of about 20 keV post-acceleration at the end of the flight tube. The purpose of this modification was to increase the detection sensitivity for large mass ions. These particles otherwise hit the conversion dynode of the electron multiplier detector with relatively low velocity resulting in poor conversion. Laser desorption of large ($m/z > 10,000$) (Karas and Hillenkamp, 1988) and very large ($m/z > 100,000$) (Tanaka et al., 1988, Karas et al., 1989a) ions generated distinguished interest among mass spectroscopists who have been struggling with the volatilization problem already for more than a decade.

Due to the extended mass range, several important classes of molecules (proteins, certain polymers) became available for mass spectrometric analysis and investigation. A typical example of β -D-galactosidase MALD spectrum is shown in Fig. 1. Better preparation procedure and the introduction of new matrices yielded even higher upper mass limits reaching $m/z \approx 250,000$ Dalton (Karas et al., 1989b).

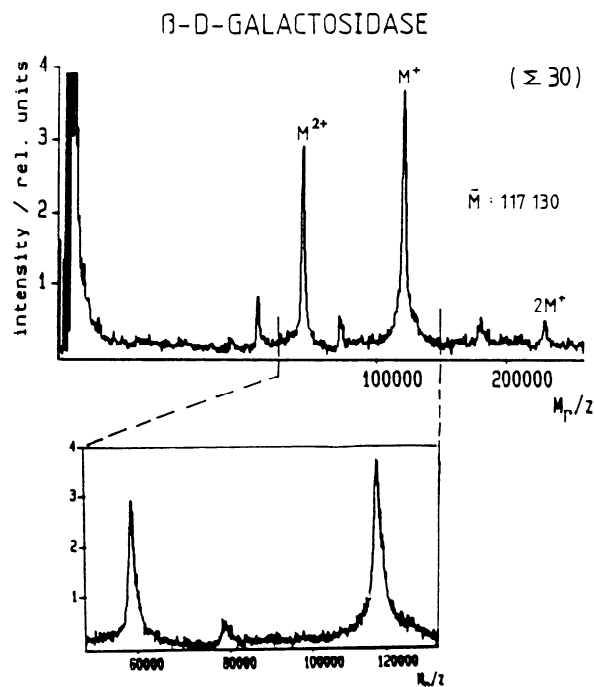


Figure 1. High mass region matrix assisted laser desorption (MALD) mass spectrum of β -D-galactosidase enzyme. Signal averaging improved the signal to noise ratio (30 shots) (Karas et al., 1989b).

These findings had a considerable impact on the pace of laser desorption investigations. In Fig. 2. we show, how the number of submitted articles related to MALD increased during the eighties. Clearly, the num-

ber of articles shows exponential growth after the discovery of MALD.

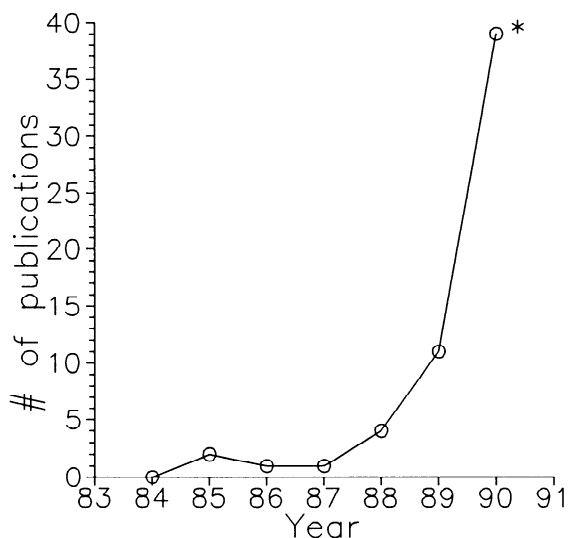


Figure 2. The number of publications related to matrix assisted laser desorption in different years. (The year of manuscript submission was considered.) * - only the first half of the year.

A growing number of groups joined the investigations (Beavis and Chait, 1989b; Nelson et al., 1989; Salehpour et al., 1989; Ens et al., 1990; Frey and Holle, 1990; Hettich and Buchanan, 1990; Nuwaysir and Wilkins, 1990; Spengler and Cotter, 1990; Vertes et al., 1990a; Zhao et al., 1990). Detection limits ranging from 5 pmol to 50 fmol and mass accuracy of about 0.1 % were established (Karas et al., 1989c). Both characteristics, however, showed strong sample dependence. Beavis and Chait introduced cinnamic acid derivatives as new matrix materials showing similar or superior features to nicotinic acid (Beavis and Chait, 1989a). Also negative ion spectra of proteins were observed (Beavis and Chait, 1989b; Salehpour et al., 1989). High mass glyco-proteins and hydrophobic proteins not accessible for plasma desorption mass spectrometry showed encouraging response in MALD experiments (Salehpour et al., 1990).

Mass accuracy of the MALD method was squeezed to ± 0.01 % by using internal calibration of the mass scale (Beavis and Chait, 1990a). In most cases, the required sample amount (0.5 to 1 μ L of sample solution containing several pmol or several hundred fmol analyte) compared favorably with the sample requirements of other mass spectroscopic techniques.

Frequency tripled Nd-YAG laser ($\lambda=355$ nm) was successfully tried to demonstrate that MALD is feasible with more affordable nitrogen lasers ($\lambda=337$ nm) (Beavis and Chait, 1989c). Recently, possibilities have been

broadened even further by the experiments on MALD using infrared radiation of a Q-switched Er-YAG laser ($\lambda=2.94$ μ m) (Overberg et al., 1990) and of a CO₂ laser ($\lambda=10.6$ μ m) (Hillenkamp, 1990). The relatively high price tag on laser equipment made these findings especially remarkable. As a consequence, a number of groups equipped already with any of the indicated lasers can join the studies.

The other direction of extending the method's possibilities was the search for appropriate and better matrices. In a thorough study Beavis and Chait tested about fifty different matrix materials (Beavis and Chait, 1990c). Using frequency quadrupled Nd-YAG laser ($\lambda=266$ nm) the best results in peptide volatilization and ionization could be obtained by applying nicotinic acid, pyrazinoic acid, vanillic acid, ferrulic acid, sinapinic acid, caffeic acid and certain other cinnamic acid derivatives. Nitrobenzyl alcohol on a fibrous paper substrate turned out to be a useful matrix as well (Zhao et al., 1990). In addition to the matrices used in the UV experiments carboxylic acids, glycerol and urea proved to be also useful in the infrared experiments. Low analyte specificity and moderate volatility in the vacuum system are among the desirable features expected from prospective matrices.

Experiments aimed at the better understanding of the physical background of the MALD phenomena were launched at several laboratories. Initial kinetic energy distributions of MALD generated ions were measured utilizing pulsed ion extraction in a Wiley-McLaren type time of flight instrument (Spengler and Cotter, 1990). Departing ion energies showed about 1 eV mean value. The question of substrate participation was revisited in a study, where the laser and the ion optics were situated on opposite sides of the suspended sample crystals (Vertes et al., 1990a). Successful matrix assisted experiments on different low mass peptides in this transmission geometry supported the idea of negligible substrate participation in the case of a strongly absorbing matrix.

Several groups were able to establish laser irradiance thresholds in ion generation (Hedin et al., 1990; Ens et al., 1990). This phenomenon has been reported many times earlier in the general context of laser ionization. Although the absolute numbers for the irradiance vary from study to study or even from sample to sample, the values are generally situated in the vicinity of several times 10^6 W/cm². The uncertainties are mainly due to poorly defined light intensity measurements and to uncontrolled variations in sample morphology. Using single ion counting the irradiance threshold of insulin ion generation and a narrow range irradiance dependence of ion yield were determined (Ens et al., 1990) (see Fig. 3). With the analysis of pulse height distributions they also inferred that MALD is the result of a collective effect.

Preliminary results were reported on the metastable decay of MALD generated ions. 354.5 nm laser desorption of insulin molecules resulted in molecular ions decaying in the field free flight tube, loosing probably a

relatively small neutral entity (Beavis and Chait, 1990e). Beavis and Chait also concluded that metastable decay is an indication of high internal (vibrational) temperatures of the desorbed ions.

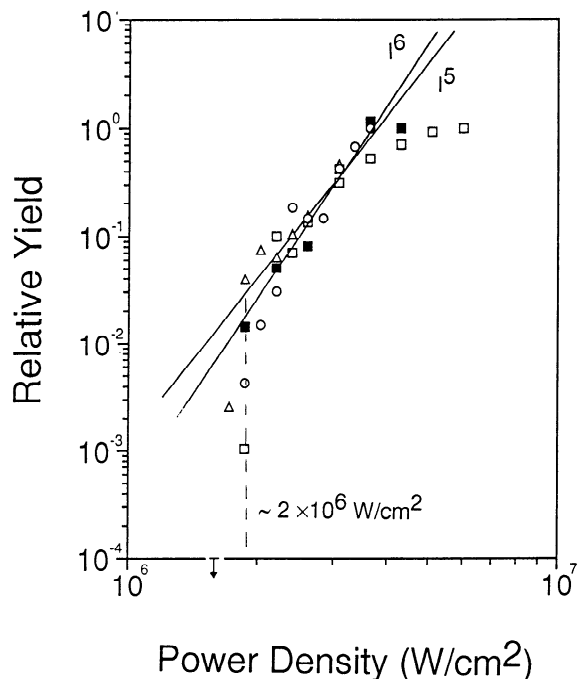


Figure 3. Irradiance dependence of the insulin molecular ion yield clearly exhibit threshold behavior (Ens et al., 1990). The different symbols correspond to different detector openings.

Desorbed neutrals and molecular ions were investigated using tunable UV laser light for desorption, supersonic jet cooling and resonance enhanced multi-photon ionization (REMPI) (Frey and Holle, 1990). It was instructive to compare the effect of matrix assistance and supersonic cooling on the fragmentation patterns. Without cooling and without matrix no molecular ion signals were observed for gramicidin D ($M_w=1881$), whereas the introduction of either jet cooling or matrix assistance alone was sufficient to suppress the fragmentation. Preliminary estimates of neutral velocity distributions indicate about 300 K translational temperature, independent of laser wavelength ($\lambda = 10.6 \mu\text{m}$ or 266 nm).

Pilot experiments to utilize MALD in Fourier-transform mass spectrometers (FTMS) have been described (Hettich and Buchanan, 1990). Low mass ions ($m/z < 2000$) were exhibiting strong matrix enhancement under FTMS conditions, but efforts to detect molecular ions in the higher mass region were unsuccessful. The origin of this deficiency was linked to the detection limitations of the Fourier-transform instrument.

Another attempt to combine MALD with FTMS concluded that the nicotinic acid matrix material sublimed from the probe tip within 15 minutes under the usual vacuum of FTMS measurements (10^{-8} torr) (Nuwaysira and Wilkins, 1990). Possible alternatives for matrix material were tested with encouraging results in the case of sinapinic acid and sucrose.

Recently, a different scheme of laser volatilization has been introduced (Nelson et al., 1989, Becker et al., 1990). Thin films of the aqueous analyte solution were frozen onto cooled metal probe tips. The laser radiation ablated the ice film. The volatilized material was either collected on a solid surface and subsequently analyzed using gel electrophoresis or directly analyzed by time of flight mass analysis with or without postionization. Electrophoresis results on DNA digest showed very high mass particles ($m/z \approx 6$ MDa) in the plume, whereas TOF detection revealed $m/z \approx 18,500$ ions (Nelson et al., 1990a). These results were rationalized in terms of sudden heating of the metal surface followed by fast heat transfer to the ice layer and by explosive boiling (Nelson and Williams, 1990b).

Applications of MALD MS are just beginning to mushroom. The most important achievements are expected on areas where the unique features of the method can be utilized. The unparalleled mass accuracy in the high mass region made it possible to revise the calibration curves of certain commercial gel electrophoresis molecular weight marker kits (Kratzin et al., 1989). High mass capabilities are exploited in the studies of monoclonal antibodies (Siegel et al., 1990a and Siegel et al., 1990b) and in the investigation of large enzyme molecules (Hillenkamp and Karas, 1990). Another beneficial feature was emphasized in an experiment where the mass spectrum of commercial bovine milk was taken (Beavis and Chait, 1990b). This exercise intended to demonstrate the potential to analyze proteins in unpurified biological fluids, which are complex mixtures of organic and inorganic constituents (Beavis and Chait, 1990d). Physiological salt concentrations — usually prohibitive for MS methods — were not detrimental on MALD spectra (Karas et al., 1990).

Other groups of bio-organic compounds such as oligonucleotides and carbohydrates also exhibit spectral responses, although for the time being only in the lower mass region ($m/z < 32,500$; $m/z < 25,000$ and $m/z < 4,000$ respectively) (Karas et al., 1990).

Fast development is expected in the near future in the diversification of applications, although, the inertia of standard biochemical approach is immense. Molecular weight determination of large biomolecules is routinely done as part of the gel electrophoresis separation step. It is difficult to compete in price and in ease of operation with the conventional method, especially, since the introduction of pre-cast gels considerably shortened the analysis time, as well. MALD will have to offer substantial superiority in certain features, in order to get widespread recognition. The price of the instrumenta-

tion will still be prohibitive for many laboratories but centralized handling of the tough problems in specialized facilities seems feasible.

Proposed mechanisms and models

Mechanisms for the desorption of large molecules by fast ion excitation have been reviewed recently (Johnson, 1987). Because of similarities between fast ion desorption and laser desorption of biomolecules and because the former has a longer history there are plenty of fertilizing ideas to be transferred. However, here we choose a different approach.

There are two major questions to answer in connection with the laser induced volatilization of large molecules.

- a/ What is the nature of the laser induced process leading to the transition from solid to gas phase?
- b/ How can large molecules escape fragmentation in an environment abruptly energized by the laser pulse?

These questions are equally relevant both in LITD and in MALD experiments. The rough options to answer question a/ are to invoke thermal or electronic processes, but closer inspection of the problem has led to suggesting several different mechanism falling within these categories. Question b/ is specifically emphasized in the MALD situation where the matrix ions may undergo extensive fragmentation whereas the embedded large molecules desorb intact.

Extremely fast energy deposition — as it is the case with laser heating — generates a strongly non-equilibrium population of energy levels in the solid. There are numerous different regimes for the relaxation of this situation. We enlist here certain possibilities which involve volatilization and have shown up in the explanation of related phenomena.

(a/i) *Spinodal decomposition.* It is known from thermodynamics that condensed phases can only be superheated up to a point where homogeneous vapor nucleation becomes dominant over heterogeneous nucleation (evaporation) and the whole phase is suddenly transformed into vapor (von Alleman, 1987). A rough estimate of the corresponding temperature is $0.9 \times T_c$ where T_c is the critical temperature. This phenomenon — sometimes called phase explosion or spinodal decomposition — was referred to in the explanation of fast atom bombardment (FAB) ionization experiments (Sunner et al., 1988). Recently, preliminary molecular dynamics simulations have shown the feasibility of the spinodal decomposition mechanism in particle and laser desorption experiments (Shiea and Sunner, 1990). Molecular dynamics simulation of the model is capable of visualizing strongly non-equilibrium phase transitions induced by high energy projectiles in two dimensional Lennard-Jones fluids. Extension of the calculations to three dimensions and to laser excitation in MALD experiments are expected.

(a/ii) *Cool plume.* Hydrodynamic description of

laser generated plume expansion has contributed to the insight of laser solid interaction (Vertes et al., 1988a, Vertes et al., 1990b), especially in the neighborhood of the plasma ignition threshold (Vertes et al., 1989a). Shock wave development under moderate and high irradiance conditions was demonstrated and kinetic energy distributions of desorbed and ablated ions were successfully accounted for (Vertes et al., 1989c). A refined version of the one-dimensional model was able to handle phase transitions, surface recession and heat conduction processes in the solid, and electron-neutral inverse Bremsstrahlung light absorption, multiple ionization and radiation cooling in the plume (Balazs et al., 1990). The adaptation of this model to typical conditions of MALD experiments provided encouraging preliminary results (Vertes et al., 1991). A frequency quadrupled Nd-YAG laser pulse acted on a matrix surface at 10^7 W/cm² irradiance. As a result, the surface temperature was temporarily elevated somewhat above the sublimation temperature releasing, therefore, a certain amount of vaporized matrix material. The remarkable feature of this vapor cloud was that the expansion cooling overcompensated the laser heating, and the plume temperature dropped below 200 K. In this way the plume acted as a jet-cooling device, possibly contributing to the lack of fragmentation in the case of the embedded molecules. This model is able to account for the suitability of matrices with low sublimation temperatures, but the explanation of matrix fragmentation is not straightforward.

Measuring laser induced thermal decomposition processes in thermally extremely labile substances without and in the presence of nicotinic acid matrix indicated the participation of a cooling mechanism if the matrix was present (Claereboudt et al., 1991). Aryltriphenylphosphonium halide guest molecules were used as "molecular thermometers" in typical MALD experiments. The mass spectra revealed no thermal decomposition and low internal energies of the guest molecules and therefore supported the cool plume model.

(a/iii) *Phonon avalanche.* Fain and Lin treated UV laser induced non-selective desorption theoretically (Fain and Lin, 1989a, Fain and Lin, 1989b). In their model the adsorbates (CH₂I₂ on Al₂O₃, Ag or Al) are excited to higher electronic states. The excitation energy is then converted into internal vibrations of the adsorbates and subsequently to phonons. They showed that a system of excited anharmonic oscillators can become unstable under the influence of some external force field. If the energy dissipation from the phonon modes is slower than their energy gain the number of phonons shows exponential divergence or in other words a phonon avalanche can be observed. The main characteristics of the model are the existence of a surface coverage dependent threshold laser fluence for the avalanche and the molecular non-selectivity of the above-threshold desorption. On the other hand the below-threshold regime of their model predicted selective desorption. Similar treatment of IR laser induced desorption exhibited nei-

ther molecular selectivity nor threshold behavior and no phonon avalanche was reported (Fain et al., 1989).

(a/iv) *Desorption induced by electronic transitions (DIET)*. Low energy electrons or UV photons may excite the surface adsorbate complex to a repulsive antibonding electronic state. A possible relaxation of this excitation is the departure of the adsorbate from the surface. This mechanism of desorption has been discovered and rediscovered several times (Menzel and Gomer, 1964, Antoniewicz, 1980). A relatively new development in the utilization of the DIET model was its application to bulk etching of organic polymers by far UV radiation (Garrison and Srinivasan, 1984, Garrison and Srinivasan, 1985). In the framework of this DIET model it was possible to rationalize polymer ablation without melting or any other thermal effect. In the irradiated volume of the polymer the monomer units were thought to be instantaneously excited to the repulsive state and leave the bulk of the solid with coherent motion. Concerning the applicability of this model to MALD, the recently demonstrated possibility of IR MALD seems to be a clear contraindication.

It is straightforward from the arrangement of LITD and MALD experiments that laser energy deposition occurs mostly to anything but the large molecules. In the case of LITD the solid substrate acts as an absorber whereas in MALD experiments the matrix molecules are to be energized. The answer to question b/ is related to this separation of energy deposition from the large molecules.

(b/i) *Vibrational energy transfer experiments*. Intramolecular and intermolecular vibrational energy transfer in condensed phases has been studied by picosecond laser spectroscopic methods (Seilmeier and Kaiser, 1988). These investigations are aimed at the understanding of vibrational energy redistribution and equilibration after ultrashort light pulse excitation of 10 ps duration and less. Large organic molecules (usually dyes) dissolved in small molecule organic liquids exhibit the following features:

- If excited above 1000 cm^{-1} they often redistribute the excess energy over the vibrational manifold of the electronic ground state with a relaxation time smaller than 1 ps. It has also been shown that the transient internal temperature of the excited molecule is a meaningful term.
- Intermolecular energy transfer is also very fast and has a relaxation time of about 10 ps, largely dependent on the degree of excitation and on the solvent molecules.
- Excitation to high vibrational states of the S_0 ground state may exhibit fast relaxation to the bottom of the first electronically excited state (S_1). Vibrational redistribution of the energy in S_0 and in S_1 has relaxation times in the order of 0.5 ps.

Similar studies were carried out on surface adsorbates too (Heilweil et al., 1989).

From our point of view these investigations should have a slightly different emphasis. First of all, in LITD and in MALD the energy is dumped into the solid substrate and into the matrix, not into the large molecule. Therefore, a reverse flow of energy is generated and should be studied. Second, the amount of deposited energy is deliberately set to levels where desorption and/or phase disintegration occur, therefore, highly anharmonic displacements are induced. Nevertheless, we cited the studies above, because they indicate the possibility of time resolved energy transfer experiments of similar nature. Scarce reports of vibrational temperature measurements of molecules after laser evaporation from a cryogenic matrix show vibrational cooling down to $T=170\pm 30\text{ K}$ (aniline in CO_2 matrix, Nd-YAG laser, $3\times 10^8\text{ W/cm}^2$) (Elokhin et al., 1990). Under different conditions, similar temperatures were also indicated by the *cool plume model* (Vertes, Irinyi and Gijbels, work in progress).

(b/ii) *Desorption vs. fragmentation in LITD*. There is a long history of investigating desorption and fragmentation kinetics at different heating rates (Beuhler et al., 1974, Deckert and George, 1987). The microscopic dynamics of energy transfer from a rapidly heated surface to the adsorbate species has been treated by stochastic trajectory modeling on a computer (Lucchese and Tully, 1984, Lim and Tully, 1986) by classical molecular dynamics simulation (Holme and Levine, 1989) and by the evaluation of the survival probability of adsorbates without degradation (Muckerman and Uzer, 1989, Zare and Levine, 1987). All these studies arrive at similar conclusions: if the heating of the surface is rapid enough the desorbing species may have considerably lower temperature. In the case of frequency mismatch between the physisorption bond and the chemical bonds of the adsorbate an energy transfer bottleneck is formed, for the surface phonons pump readily only the adsorption bond (Zare and Levine, 1987). The bottleneck model in its original form was suitable to explain LITD but not MALD experiments.

(b/iii) *Popcorn model*. Another approach, stemming from high energy particle induced desorption was based on the rapid vibrational excitation of the surface sample layer including the embedded large molecules (Williams and Sundqvist, 1987). According to this picture, vibrational excitation is accompanied by mechanical expansion on the picosecond timescale. This expansion detaches particles from the surface layers by pushing the expanding molecules against the substrate and generating reaction momentum. This model suggests an interesting way of converting internal excitation of molecules to their translation. It is worthwhile to consider this mechanism to explain MALD, however, one can expect difficulties in accounting for matrix fragmentation and in describing the role of the matrix.

(b/iv) *Homogeneous bottleneck model*. This description has been an extension of the bottleneck model introduced for LITD to the MALD situation (Vertes et

al., 1990c, Vertes and Levine, 1990). The laser energy is adsorbed by the matrix in the same phase where the large molecules are. As a consequence, the energy transfer is from the matrix molecules towards the embedded large molecules and the bottleneck is posed by the coupling hydrogen bonds between them. Two types of phase disintegration mechanisms were considered: sublimation and mechanical fragmentation. A simple competitive kinetic model was able to reproduce several experimental findings: the existence of a laser irradiance threshold and its estimated value, the suitability of matrices with low phase transition temperature, the low volume concentration requirement for the large molecules, the need for a short laser pulse, i.e. for fast heating rates. It was also predicted that sample cooling would extend the possibilities of the method. A generalization of the idea involved in MALD suggests that new methods can be successful, if the liberation of large molecules by phase disintegration precedes their destruction by fragmentation (Vertes, 1990).

There are unanswered questions in all the models discussed above. These models do not account for ion formation, – a prerequisite for mass analysis and detection in mass spectrometry. Further shortcoming of these mechanisms that neither of them explain the large differences in efficiency between matrices with similar phase transition temperatures and optical characteristics (Beavis and Chait 1990c).

Conclusions

It is apparent from the survey of laser desorption literature, especially since the fertilizing discovery of new sample preparation techniques that the method has great potential for the analysis of large biomolecules. The virtues of laser desorption put it into the forefront of organic mass spectrometric techniques, together with electrospray ionization. At present, it is not possible to establish superiority of one of these ion sources, rather we have to regard them as complementary possibilities of analysis.

There are several suggested mechanisms and a number of different models for the description of physical phenomena involved in laser desorption. The volume and the accuracy of experimental findings is not enough at the moment to exclude or disprove any of them with certainty. However, a general feature of all the concepts can be extracted already. Successful laser desorption of intact large molecules seems to show the repetitive pattern of restricted energy transfer which prevents excessive energy flow into the desorbing species.

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Discussion with Reviewers

M. Karas and U. Bahr: Major limitations are given for all the models if direct conclusions for the matrix laser desorption-ionization (LDI) process are to be drawn. These are mainly: All models in principal deal only with the process of setting free neutral molecules. With regard to MS — which has to rely on ions — this is only one, though important part of the coin. Equally important is the ionization process, it gives the prerequisite for mass spectrometric detection. And with respect to the matrix-LDI process this is still not the whole story,

as the formation of a uniform solid solution of the analyte molecules within the crystalline matrix is another decisive prerequisite for a successful matrix-LDI.

P. Williams: You fail to distinguish between volatilization and ionization. Thus matrices which don't produce protein mass spectra may still efficiently ablate and eject *neutral* protein molecules. Ionization is a separate topic, and using mass spectral results alone to draw conclusions about the process of molecule ejection can be misleading.

Authors: We fully agree with both comments. Indeed, ionization is a separate and very important topic. The reason we did not deal with this problem is the almost complete lack of reliable experiments and theoretical models addressing the ionization step separately. We even tried to emphasize the limited scope of this tutorial in the title speaking only about laser desorption. To the best of our knowledge there are, however, a few studies reporting on laser ionization mechanisms (Heinen, 1981, van der Peyl et al., 1982b, Chiarelli and Gross 1987 and Viswanadham et al., 1988). Preliminary calculations of ion-molecule reaction rates in laser generated plumes (Vertes et al., 1989d, Vertes 1990) and of the effect of symmetrical charge transfer processes are also available (Vertes et al., 1988b) in the literature.

M. Karas and U. Bahr: The discussion of the polymer ablation was — to the knowledge of the referees — never explicitly done within the "DIET-model". In any case most of the earlier considerations seem to be obsolete now, experiments and discussion in the meantime have gone much further, too many contradictions have ruled out the early model [especially the finding that the majority of the ablated material is still polymer (ca. 2500 Da) rather than molecular]. Now the process is described rather as a macroscopic one (shock waves, subsurface boiling and volcano-like ejection of material).

Authors: Polymer ablation is an intensively studied area and involves different mechanisms depending on the laser and on the nature of the polymer. For example, — in contrast to the statement of the reviewer — in a quite recent report on ultraviolet laser ablation of polyimide films we find support for almost complete fragmentation: "The ablation process must involve many photons per monomer unit to account for the production of predominantly small (< 4 atoms) products and the ejection of these fragments at supersonic velocities." (Srinivasan et al., 1987). Furthermore, we are not interested in the validity of the DIET-model for the polymer ablation situation but suggest this mechanism for further consideration with respect to MALD studies.

P. Williams: How fast should "fast heating" really be to allow ejection to prevail over fragmentation? If intramolecular relaxation occurs on a picosecond timescale, then, even with a bottleneck, are irradiation times 4-5 orders of magnitude longer (10 - 50 ns) really short enough to preclude intermolecular energy transfer?

Authors: In the first presentation of our homogeneous

bottleneck model we hoped to demonstrate that 10 ns irradiation time is short enough to avoid substantial heating of the guest molecules during their desorption period (Fig. 2. in Vertes et al., 1990c). We also showed that a 50 ns laser pulse results in somewhat warmer desorbing molecules but may lead to extensive thermal degradation in the solid phase (Fig. 3. of the same article). In the light of more recent results demonstrating that even 200 ns Er-YAG laser pulses produce intact ions of large guest molecules (Overberg et al., 1990) the predicted 50 ns upper limit seems short, indeed. We shall note, however, that the combination of an energy transfer bottleneck in the solid phase and expansion cooling in the gas phase may still explain the success of longer pulses.

P. Williams: Recent results indicate that MALD works best at low irradiances where only a fraction of the matrix molecules in the irradiated volume absorb a photon. These may then dissociate, which is an endothermic process, and the surrounding matrix molecules may act to shield the analyte by absorbing and dissipating the residual energy of the dissociation products. In such a case is the bottleneck model even necessary?

Authors: The bottleneck model may not be necessary, but it seems feasible. We are not convinced, however, about the feasibility of the scenario suggested by the reviewer. Taking the typical threshold irradiance as 5×10^6 W/cm² and the typical absorption coefficient as 4×10^4 cm⁻¹ (nicotinic acid) the number density of the absorbed 266 nm UV photons in a $\tau=10$ ns laser pulse is about $\tau \alpha I_0 / h\nu = 3 \times 10^{21}$ cm⁻³. This value is almost half of the number density of solid nicotinic acid: 7×10^{21} cm⁻³. In other words, almost every second nicotinic acid molecule absorbs a laser photon in contrast to the hypothesis of the reviewer.