D. G. Howitt, Ed., *Microbeam Analysis — 1991*

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THE COOL PLUME: HYDROCYNAMIC DESCRIPTION OF MATRIX-ASSISTED LASER DESORPTION (MALD)

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Matrix-assisted laser desorption (MALD) as a soft volatilization and ionization technique was introduced three years ago.[1,2] Since then it acquired a reputation as a strong candidate for the analysis of high-molecular weight biopolymers. MALD remained in competition with electrospray ionization for the availability of a similar mass range and for their comparable detection limits. In other respects (e.g., the problem of interferences and coupling to separation techniques) they are considered to be complementary methods. However, in the atmosphere of excitement caused by the vast field of potential new applications, not too much attention has been paid to answering basic questions concerning the mechanism of the volatilization and ionization phenomena.

The experiment itself is relatively simple. Solutions of the large (guest) molecules and of the matrix (host) material are mixed, providing 1:1000 to 1:10 000 molar ratios. A droplet of this mixture is dried on metallic substrate and excited by a laser pulse in vacuum. The generated ions are typically analyzed by a time-of-flight mass spectrometer. The astonishing finding was that extremely large molecules (molecular weight exceeding 100 000 Da) could be transferred to the gas phase and ionized by this method.

Some early efforts were directed toward broadening the range of applicable matrices and laser wavelengths and resulted in a rough outline of required matrix material properties.[3] A promising candidate for a successful matrix should exhibit the following features:

(a) strong light absorption at the laser wavelength;
(b) low volatilization temperature (volatilization shall take place preferably in the form of sublimation);
(c) common solvent with the analyte; and
(d) ability to separate and surround the large molecules in a solid solution without forming covalent bonds.

The first systematic measurements describing several details of the desorption process have been reported only recently.[4] The most important findings included the establishment of accurate threshold irradiances and the revelation that the underlying process is collective in nature. Experiments in alternative configurations showed that the presence of a solid substrate is not indispensable and thus opened the possibility of performing MALD experiments in transmission geometry.[5] It was also shown that mass spectrometry is not the only method to detect the large molecules.[6]

Theoretical investigations date back to efforts devoted to the understanding of plasma desorption and other high-energy-particle-induced desorption techniques.[7] An important basic question for all these methods is, how can we account for the transfer of large molecules to the gas phase without fragmentation or degradation? The intriguing similarity between MALD and the other soft ionization methods is that they all start with sudden energy deposition and they all yield large molecules in the gas phase. Energy deposition and redistribution processes seemed to be a key factor in the description of MALD mechanisms.[8,9] Attempts have also been made to account for the energy transfer to the large molecules during their volatilization.[8,9] A possible reason for the lack of degradation of these molecules is the presence of an energy-transfer bottleneck due to frequency mismatch between lattice vibrations in the solid and intramolecular vibrations in the large molecules.[10,11]

In this paper we outline a scenario in which the laser energy deposited in the solid matrix leads to heating and phase transition. The generated plume, in turn, undergoes gasdynamic expansion and exhibits strong cooling. The entrained large molecules are therefore also cooled and stabilized in the expansion. First we estimate the threshold irradiance to achieve phase transition in the matrix. In the second part of this contribution we also report preliminary hydrodynamic calculations of the plume expansion to check the feasibility of this idea.

Irradiance Threshold for Plume Formation

There are several regimes of laser-solid interaction for a given material, depending on the laser irradiance. The simplest case involves the following possibilities:

(a) surface heating with thermal desorption;
(b) surface melting with surface evaporation;
(c) volume evaporation;
(d) formation of optically thick plume;
(e) plasma absorption in the plume; and
(f) optical breakdown (in transparent insulators).

The separation of these regimes according to the principal determining factors (i.e., laser irradiance I_0 and solid optical absorption coefficient α) are shown in Fig. 1. The three different thresholds (volatilization threshold I_0^{volat}, plasma ignition threshold I_0^{plasm}, and optical breakdown threshold I_0^{break}) are marked by dashed lines. Although their values are

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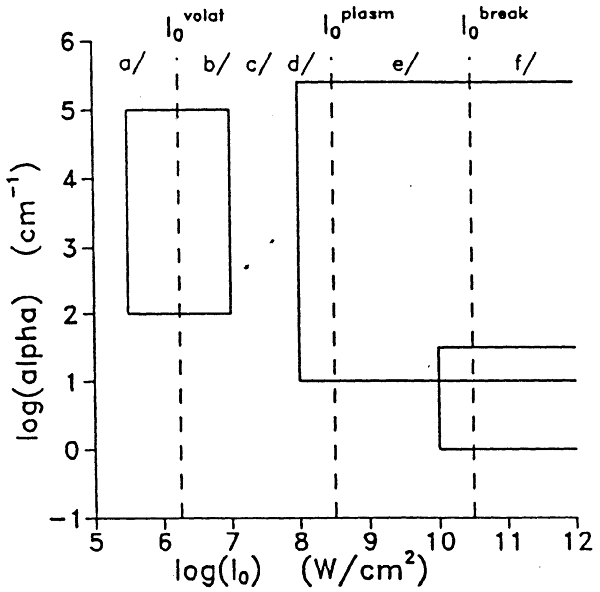

FIG. 1.--Various regimes of laser solid interaction are roughly divided according to laser irradiance and optical absorption coefficient.

well defined, shifts can be expected in a given experiment according to the choice of laser or target material.

In an earlier study we dealt with the transition from normal absorption of the laser-generated plume to plasma absorption characterized by 10^8-10^9 W/cm^2 threshold irradiance.[12] For the high-irradiance transition between regimes (d) and (e), the threshold was established on the basis of the investigation of normal absorption vs plasma absorption.

Matrix-assisted laser desorption is a much milder process, which typically requires about 10^6 W/cm^2 irradiance and low-melting-point or low-sublimation-temperature materials. Recently, it has been shown by elaborate irradiance threshold measurements that the underlying process is a collective effect, similar in principle to phase transitions.[4] Furthermore, threshold irradiance values change little when the mass of the guest molecules is changed over an order of magnitude.[13] Therefore, we assume that the threshold of our concern marks the transition between regimes (a) and (b) or between processes (a) and (c). To get an estimate of the required irradiance, we set the following condition. In order to reach volatilization, the elevated surface temperature due to laser heating must exceed the melting or sublimation temperature of the matrix:

$$T_{surf} \geqslant T_{subl} \tag{1}$$

The rise of the surface temperature $\Delta T_{surf}(t)$ at time t under the influence of a uniform penetrating light source with I_0 irradiance is:[14]

$$\Delta T_{surf}(t) = I_0\{\delta/\pi^{\frac{1}{2}}\} - [1 - \exp(\alpha\delta/2)^2\, \mathrm{erfc}(\alpha\delta/2)]/\alpha\}/K \tag{2}$$

where $= 2(\kappa t)^{\frac{1}{2}}$; the thermal diffusivity $\kappa = KV_m/c_p$; K, V_M, and c_p are the thermal conductivity, the molar volume, and the specific heat of the material, respectively. Although, analytical formulas are shown to overestimate the temperature jump in pulsed surface heating at the irradiances of our concern ($<5 \times 10^7$ W/cm^2) the error is negligible.[15]

If we substitute for the values for material parameters for nicotinic acid (α_{265} 4×10^4 cm^{-1}; c_p = 150 J/mol K; V_M = 83.5 cm^3/mol; K = 2×10^{-3} W/cm K), the end of a frequency-quadrupled Nd-YAG laser pulse (t = 10 ns; I_0 = 10^6 W/cm^2), the surface temperature rise if $\Delta T_{surf}(t)$ = 202 K. This value (if superimposed on the room temperature) compares extremely well with the sublimation temperature of this matrix, T_{subl} = 236 C. Indeed, detailed investigations show the threshold irradiance of ion generation with MALD for both nicotinic acid[16] and sinapinic acid[4] matrix to be around 10^6 W/cm^2. The most successful matrices, their melting points, and their light-absorption characteristics are listed in Table 1. Other quantities that affect Eq. (2) (such as K, V_M, and c_p) show little variation for these materials. Based on these data and on the evaluation of Eq. (2) it seems understandable that all the listed matrices have similar threshold irradiances.

Thus, we may infer that the phase-transition scenario we put forward in the introduction does not contradict the energy-deposition estimates of this section. In the next section we try to describe the processes in the generated plume with special emphasis on plume temperatures, for this is the parameter most relevant to the fate of the entrained large molecules.

Plume Hydrodynamics

Laser-generated plume expansion has been described earlier for a variety of conditions.[18-20] In general, we are interested in the density, temperature, and velocity distributions of the laser-generated plume as they develop with time. In contrast to the laser plasma generation experiments, we expect moderate temperatures during the volatilization of the low-sublimation-point matrices.

In the course of laser-solid interaction, two distinct phases can be recognized. The first phase covers the period when the surface of the solid does not reach the phase transition temperature. In this regime material transport can be neglected and the description only accounts for generation of a hot spot on the solid surface. The temperature distribution is governed by the relation between the laser heating and cooling of the spot by heat conduction:

$$\partial[\rho e]/\partial t = -\partial[\kappa\partial(\rho e)/\partial z]/\partial z + \alpha_{solid}I \tag{3}$$

where ρe and α_{solid} stand for the energy density and absorption coefficient of the solid material. We have already seen a special solution of this equation for the case of a uniform penetrating source, Eq. (2). Because we now allow for both phase transitions in the solid

TABLE 1.--Melting temperatures and wavelengths and molar extinction coefficients of UV absorption maxima for most successful matrices used in laser desorption of peptides and proteins.[17]

Laser Desorption Matrix	T_m (°C)	λ_{max} (nm)	ϵ_{max} (L/mol·cm)
Nicotinic acid (3-pyridinecarboxilic acid)	236	266	3400
Pyrazinoic acid (2-pyrazinecarboxilic acid)	225	267	7820
Vanillic acid (4-hydroxy-3-methoxy-benzoic acid)	214	259	11900
Sinapinic acid ((3,5)-dimethoxy-4-hydroxy-(trans)cinnamic acid)	192	240	10000
Caffeic acid ((3,4)-dihydroxy-(trans)cinnamic acid)	225	297 235	?
Ferrulic acid (4-hydroxy-3-methoxy-(trans)cinnamic acid)	174	235	?

and various laser pulse profiles, a numerical solution of this equation shall now be sought. This solution can be used afterward as a boundary condition for the description of plume expansion.

The second phase starts when the surface of the solid is heated above the phase-transition temperature. At this stage the vapor pressure of the material becomes significant and the material transport across the surface cannot be neglected. To deal with the expansion problem we must solve a simplified set of hydrodynamic equations that express conservation of mass, momentum, and energy.

$$\partial[\rho]/\partial t = -\partial[\rho v]/\partial z \tag{4}$$

$$\partial[\rho v]/\partial t = -\partial[p + \rho v^2]/\partial z \tag{5}$$

$$\partial[\rho(e + v^2/2)]/\partial t = -\partial[\rho v(e + p/\rho + v^2/2)]/\partial z + \alpha_{plume} I \tag{6}$$

where ρ, v, p, and α_{plume} denote the density, hydrodynamic velocity, pressure, and absorption coefficient of the plume, respectively. We note that all the transport equations, Eqs. 3-6, are written in one-dimensional form. Therefore, only processes along the z corrdinate (perpendicular to the surface) are accounted for and transport is neglected in the model. Assuming a Gaussian beam profile, this approximation is good in the middle of the beam, where radial gradients are vanishing. However, the relevance of the results is not altered by this restriction, because at the volatilization threshold (where most of the experimental work is done) only the center of the spot is hot enough to contribute substantially to the volatilization process.

Coupling between Eqs. (4) to (6) and Eq. (3) is provided by the Clausius-Clapeyron equation for the vapor pressure. Under laser desorption circumstances (i.e., near threshold irradiance), the plume remains optically thin; thus, the laser-absorption term in Eq. (6) can be neglected. The plume is heated only by the transfer of warm material across the interface, and is cooled by the expansion process. Due to the relatively low temperatures, thermal ionization and radiative cooling are not significant factors, either. Solutions of Eqs. (4-6) were found by a computer code developed and reported earlier.[18]

Results

Nicotinic acid/vacuum interface was investigated under the influence of a 10ns frequency-quadrupled Nd-YAG laser pulse. The temporal profile of the 10^7-W/cm^2 irradiance pulse was approximated by a square wave. Spatial distribution of plume density and temperature above the surface are shown in Fig. 2. Three time stages are depicted in order to visualize post-pulse behavior.

The spatial density profile at the end of the laser pulse (10 ns) showed monotonous decay (Fig. 2a). Subsequent cooling of the surface substantially lowered the rate of evaporation. Consequently, the density immediately above the target dropped quickly and the plume packet detached from the surface to produce a drifting and expanding plume packet (25 ns, 50 ns). We estimated the kinetic energy of the particles stemming from plume translation. The drift velocity of the plume center of mass, $V_{drift} = 3 \times 10^4$ cm/s, could be converted to kinetic energy: $E_{kin} \approx 60$ meV. This is a value quite close to the measured energies of molecules in the plume: $E_{kin} = 40$ meV (2×10^6 W/cm2, 248 nm, 13 ns excimer laser pulse, tryptophan target).[21] It is worthwhile to notice that the plume density was relatively high; at the 50ns time stage its maximum density still exceeded 1/10th of the solid density. With further expansion of the vapor cloud, its density dropped quickly. The high initial plume density has important repercussions regarding the possibility of gas phase processes. It seems possible that certain reactions are induced in this dense cloud of particles. Most important among them are protonation, alkalination, and adduct ion formation of the guest molecules. Indeed, there is clear experimental evidence that the appearance of sodium and/or potassium containing quasi-molecular ions are bound to the presence of sodium and/or potassium ion signals and to the generation of a dense plume.[22]

Another interesting feature of the plume was the spatial and temporal variation of the temperature. The surface of the nicotinic acid was heated to the phase-transition temperature, but a quick decay of plume temperature was observed with increasing distance from the surface (Fig. 2b). The actual value of the temperature drops well below room temperature due to the expansion cooling. Comparison of density and temperature distributions at 50 ns provided an estimate of 140K at the center of mass of the plume. The obvious consequence of such a cooling would be a stabilizing effect for the entrained large molecules. The situation is very reminiscent of the two laser experiments, in which jet cooling of the large molecules is usually introduced between the desorption and ionization step. Experimental verification of low plume temperatures by thermally labile molecules as molecular thermometers was reported earlier.[22]

However, simple heating of the matrix lattice by the laser pulse gives no satisfactory explanation of all the findings of UV MALD experiments. In contrast to the presence of intact guest molecules in the plume, host fragments can be abundant in the mass spectrum. This observation points to the importance of exploring the possible energy-transfer pathways in the system.[9] In the UV experiments, primary energy deposition leads to electronic excitation of the host molecules ($\pi \rightarrow \pi^*$ transition in the case of a nicotinic acid matrix). Quick internal conversion processes lead to vibrationally highly excited ground states. Part of the host molecules decompose from these vibrational states, but another part transfers its energy to the lattice. Thus, the lattice is heated and the phase transition temperature can be occasionally reached. At this stage the question arises: what prevents energy transfer to the guest molecules? According to our calculations of the energy transfer rates in this system, there is a bottleneck hindering the heating of internal vibrational degrees of freedom of the guest molecules.[10,11] This bottleneck is related to weak coupling between the lattice vibrations and the intramolecular vibrations of the large molecules.

Another important question is the origin of the ions in the system. It is clear from postionization experiments that the degree of

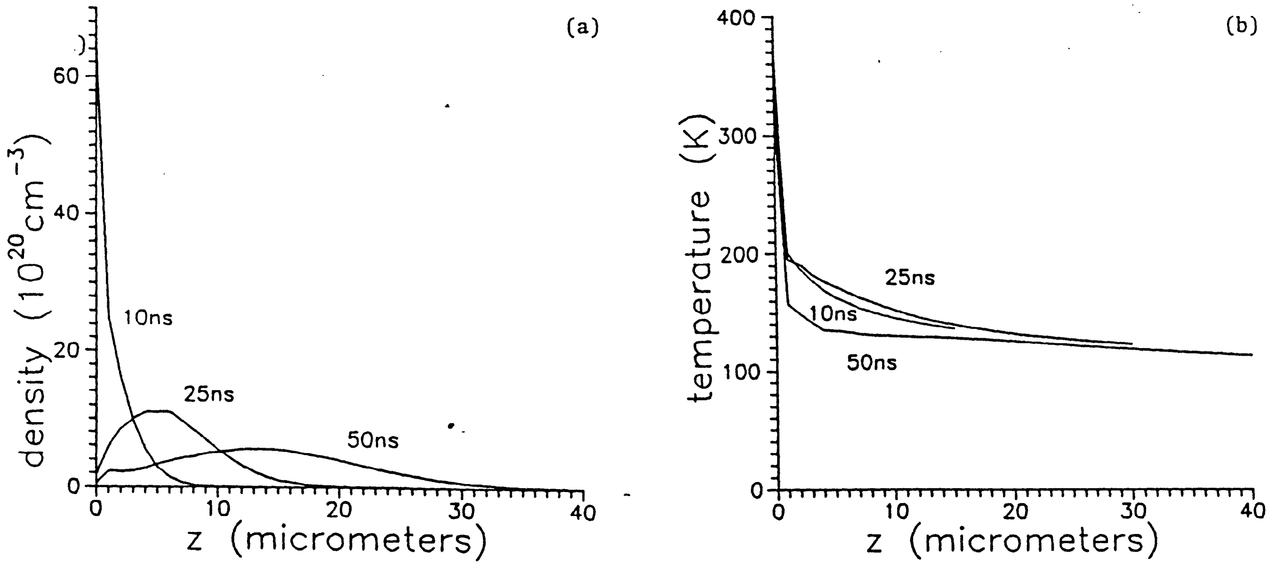

FIG. 2.--Evolution of plume above nicotinic acid surface as consequence of frequency-quadruples Nd-YAG laser pulse (10 ns; 10^7 W/cm^2). (a) Density profiles perpendicular to surface show detachment and expansion. (b) Although surface temperature reaches sublimation temperature, substantial cooling of plume is observed.

ionization in the plume is very low. In light of the calculated temperature values, it is also obvious that thermal ionization is not a feasible ion-production mechanism in these experiments. Rather, protonation and other adduct ion formation mechanisms shall be regarded as the primary source of ions. These processes can be discussed in terms of preformed ion volatilization and ion-molecule reactions. Guest particles that are already in their ionized form in the solid state (preformed ions) can give rise to gas phase ions extremely easily. Molecular ions of these substances in MALD experiments could be produced even without ion production from the matrix.[22] These findings support the suggestion that the role of the matrix is to embed and separate the guest particles in the solid phase and to entrain and cool them in the course of volatilization.

Conclusions

In this communication we suggested how to explain matrix-assisted laser desorption of intact large molecules. According to preliminary estimates of energy deposition, it seems likely that laser heating of the matrix to the phase-transition temperature is a prerequisite to carryng out successful MALD experiments. The generated plume has been described in terms of hydrodynamics and the results reflect several important features of the measurements. Most important, kinetic energies of neutral particles can be recovered. Furthermore, strong cooling of the plume is predicted, which would rationalize the absence of thermal degradation processes. Detailed calculations of plume expansion are under way to explore the effect of matrix and laser parameters on the desorption process.

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